ESTABLISHMENT OF RUMEN PROTOZOA, FUNGI AND THEIR FERMNETATIVE ROLE IN GOATS OF BUNDELKHAND

THESIS

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BY

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2005

DEDICATED

TO

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Dated 14.10.05.

Certificate

It is certified that the thesis entitled "Establishment of rumen protozoa, fungi and their fermentative role in goats of Bundelkhand" is an original piece of work done by Km. Sonu Rai, M.Sc. (Zoology) under my supervision and guidance for the degree of Doctor of Philosophy in Zoology, Bundelkhand University, Jhansi.

I, further certify that:

- It embodies the original work of candidate herself.
- It is up to the required standard both in respect of its contents and literary presentation for being referred to the examiners.
- The candidate has worked under me for the required period at Indian Grassland and Fodder Research Institute, Jhansi.
- The candidate has registered the required attendance and worked under me required period at Indian Grassland and Fodder Research Institute, Jhansi.

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Declaration

I, hereby, declare that the thesis entitled "Establishment of rumen protozoa, fungi and their fermentative role in goats of Bundelkhand" submitted by me for the award of degree of Doctor of Philosophy in Zoology, in Faculty of Science, Bundelkhand University, Jhansi, is the original piece of work done by me under the supervision of Dr. A.K.Samanta, Senior Scientist. I.G.F.RI., Jhansi and to the best of my knowledge and part or whole of this thesis work has not been submitted for any degree or any other qualification of any university or examining body in India or any university elsewhere.

S. Rai

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Chapter 1

Introduction

1. INTRODUCTION

Livestock is emerging as the driving force in the growth of Indian economy. Contribution of livestock enterprise towards agricultural gross domestic product is 25 %. Goat occupies an important place on rural economy because of its multifaceted role. On the basis of archeological evidence it is thought that the goat is first domesticated ruminant and stand second to be domesticated if considered both ruminant and non-ruminants. When man began his family operations in the dawn of history, the goat was the king- pin of the pastoral life, making possible the conquest of desert and mountain and the occupation of fertile land. Goats are potential source of meat, milk, skin, and fiber and are valued as laboratory as well as pet animal. In some parts of the country (like Bengal, Assam) people sacrifice goat to satisfy the God / Goddess. The wide range of utility combined with its docile, intelligent, graceful and frugal nature contributed to its earliest domestication. But with the advent of civilization, goats were surpassed by cattle, buffalo and sheep. The goat population is the fastest growing among the major ruminant species in spite of the fact that 41 % goats are slaughtered and 15.5 % of total goat population succumbs to natural death in the rural areas. The goat population is around 128 millions (Ranjan, 2003). Considering the annual rate of population growth (about 3.6 %), with mean slaughter rate (41 %) and mortality rate (15.5 %), this small ruminant has achieved potential growth accounting 60.1 % per year.

The importance of goat on the Indian agriculture is evidenced by its unparalleled economic traits, ability to get acclimatized under diversified agro

climatic situations, high fertility and short generation interval. From economic point of view, the goat is ideally suited for small and marginal farmers for the low cost maintenance, short-term return on capital with lower risk on capital investment. Goat thrives and contributes to rural economy even in areas where cattle and buffalo enterprises are difficult. These small ruminants are energetic, inquisitive and versatile in the art of food gathering. They have a greater tendency to change their diet with changing season than large ruminants. Semi-arid areas with sparse vegetation, bushes and shrubs can not support cattle but suit the browsing taste of goat.

Depending upon the adaptability to climatic conditions, the breeds differ in body conformation. Besides the several well defined breed of goats, certain local breeds are found in different agro-climatic zone. One of them is local breed of Bundelkhand characterized by black in colour, medium body size, Roman nose, long ear, thrive well on bushes and shrubs. Traditionally, this local breed has been a victim of neglect both by researchers and planners.

Individual microbial species that have developed in the rumen interact in a complex manner and cite some nature's best examples of microbial symbiosis. Degradation and utilization of ingested plant bio-molecules is governed by plants, animal and microbial factors (Selinger et al., 1996). Effectiveness of the rumen microbial enzymes on plant nutrition is regulated largely by the physical characteristics of plants including structure, composition, feed processing etc. Mastication, salivation and rumination by the animal initiate release of nutrients from the plant material and increase their availability to microbial digestive

enzymes. Degradation and metabolism of the feed components (cellulose, hemi-cellulose, starch, protein, lipids) by rumen microorganisms supplies the carbon, energy, amino acids and vitamins required by the host animal.

A newborn kid is just like mono-gastric animals i.e. with simple undivided sterile stomach completely free from anaerobic microorganisms. But with the growth, the all four chambers viz, rumen, reticulum, omasum and abomasums developed and the former two are for the growth and multiplication of anaerobic bacteria, protozoa and fungi. The microbiota of rumen includes 10^{10}–10^{12}/ ml bacteria, 10^{4}-10^{6}/ml protozoa (Hungate, 1966) and 10^{3}-10^{4}/ ml fungi (Orpin, 1976) of rumen liquor. The discovery of rumen protozoa in 1843 by Gurby and Delaford was the first identification regarding the presence of microbes in alimentary tract of animals. The rumen is an unusual environment for microorganisms. It is warm (39^{0}C), has low redox potential and rich in particles such as starch, plant fibers, other plant bio-molecules. During feeding there are also injections of soluble compounds such as sugars and proteins (Selinger et al., 1996). Feedstuffs consumed by ruminants are all exposed to fermentative activity of anaerobic bacteria (Sinha and Ranganathan, 1983), protozoa (Misra et al., 1996) and fungi (Orpin, 1975, Samanta and Walli, 1999) prior to gastric digestion. Dietary plant complex polymeric nutrients viz. carbohydrate, protein, lipids etc. are generally degraded by the rumen microorganisms into characteristic end products which in turn provide nutrients for metabolism by the host. Fermentation of feedstuff in the rumen yields short chain volatile fatty acids (primarily acetic, propionic and butyric), carbon dioxide, methane and

ammonia. Ruminants use the organic acids and microbial protein as source of energy and protein produced by microbial fermentation. The quantity and quality of rumen fermentation product is dependent on the types and activities of the microorganism in the rumen. The anaerobic bacteria, protozoa and fungi constitute a significant portion of hydrolytic enzyme for fibrous food digestion of the host. The rumen, the most important segment of ruminant stomach provides a physical environment and a desired supply of nutrients to support an extremely diverse microbial community. The ruminant protozoa plays diverse and important role in the metabolism as these help in degradation of cell wall and other carbohydrate constituents by secreting digestive enzymes for digestion of polysaccharides (Williams and Coleman 1988). The bacteria are most abundant in the rumen. Bacteria hydrolyze plant carbohydrates into different volatile fatty acids, succinic acid, lactic acid and ethanol. The rumen fungi attack the feed both by physical and biochemical means (Akin et al., 1989). The physical disruption of plant fiber is performed by the penetration of rhizomycelial network of the anaerobic fungus and biochemical activities is attributed by the extensive array of fiber degrading enzymes including β-1,4 endoglucanase, β-1,4 exoglucanase, β-1,4 glucosidase, xylanase, pectinase etc. (Samanta and Walli, 1995). Thus anaerobic microbial degradation of feeds helps ruminants to utilize various types of vegetation for completing their nutrition requirements. The ruminant host provides congenial atmosphere for microbial growth and multiplication. Ruminant nutritionist and microbiologists have long been interested in manipulating the microbial ecosystem of the rumen

to enhance feedstuff utilization, improve production efficiency by ruminants and alleviate problems associated with current feeding practices (Lee et al., 2000). Dietary manipulation directed towards early development of ruminal functions helps to introduce fiber diet at the early ages (Sahoo et al., 2005). This can substantially save the milk for human consumption and reduce the cost of starter ration. In India, though goat keeping is predominantly done by small and marginal farmers, but several intensive goat enterprises are coming up at different parts of the country. Therefore, the date of establishment of different microbial communities in the foregut of kid alimentary tract will be of great useful for economic goat production. Moreover, determining the quantitative contribution of different microbes in adult goat rumen will enrich the existing knowledge of rumen microbial ecosystem. Under these circumstances the following objectives were outlined:

1. To see the time of establishment of various microbial communities in the rumen of local goat.
2. To determine the role of microbes on fermentative action of rumen.

Chapter 2

Review of literature

2. REVIEW OF LITERATURE

The ruminants have ability to utilize fibrous feed material with the complex microbial population present in the rumen. The rumen is a large organ (3-15 liter in small ruminants) that accommodates digesta representing 10-20% of the body weight of the animal. Rumen temperature averages 39^0C as a result of anaerobic tissue metabolism of the ruminant and the microbial fermentation of feed materials. However, the temperature may vary 3 to 4^0C from the average due to external temperature variation and the ingestion of feed and water. Despite, copious acid production, the pH of the bulk liquid phase in the rumen of forage fed animals is maintained in the range of 6.5 to 7.0 by salivary bicarbonate, phosphate and by ammonia release during de-amination of amino acids by proteolytic and other fermentative microorganisms. A distinguishing feature of the rumen from standpoint of microbial ecology is that it is a highly anaerobic environment where reducing conditions (Eh= -150 to –35 mv) exists (Clark, 1977). Oxygen entering into the rumen during feeding and watering is rapidly consumed by very small population of facultative anaerobic microorganisms and by reaction with chemical reducing agents (Weimer, 1992). As a result, the microbial population is well protected from oxygen and strictly anaerobic bacteria, protozoa and fungi dominate the population in adult ruminants.

By virtue of the anaerobic environment, the foregut (rumen, reticulum) of goat harbor a dense and varied microbial population composed essentially of bacteria, protozoa, fungi and phage particles. Feedstuffs consumed by ruminants

are all initially exposed to the fermentative activity in the rumen prior to the gastric and intestinal digestion. The ruminal microorganisms convert dietary polysaccharide and protein into characteristic end products, which in turn provide nutrients for metablism, by the host animal. The extent and type of transformation of feedstuff thus determines the productive performance of the host. Fermentation of feedstuff in the rumen yields short chain volatile fatty acids (primarily acetic, propionic and butyric acids), carbon dioxide, methane, ammonia and occasionally lactic acid. Ruminants use the organic acids and microbial protein as source of energy and amino acids, respectively, but methane, heat and ammonia cause a loss of energy and nitrogen. The quality and quantity of rumen fermentative product is dependent on the types and activities of the microorganisms in the rumen.

The gut microbial ecosystem in different species of animals helps in the digestion process and is beneficial to the host in many different ways. The microbial community in the gastro intestinal tract of various animal species is characterized by high population density in the types of microorganisms. The major groups of microbes, which inhabit the gastrointestinal tract, include protozoa, fungi, bacteria, yeast and bacteriophage (Hobson, 1988). The rumen eco-system contains bacteria (10^{10}-10^{11} cells /ml, representing more than 50 genera), ciliate protozoa (10^{4}-10^{6}/ml, from 25 genera) and anaerobic fungi (10^{3}-10^{5}/ml) (Hobson, 1988, Mackie et al., 1996).

The role and biochemical capabilities of the rumen microbes are less fully understood. Under certain dietary conditions protozoa account for

approximately half of the ruminal biomass and therefore, substantially contribute to total volatile fatty acid production. Thus the exact role of microbes in the rumen fermentation of local goat can be estimated by studying the changes in the rumen picture of animals free from particular microbial communities.

2.1 ESTABLISHMENT OF MICROBES

Adult ruminant animals having well developed compound stomach bearing a complex micro flora consisting of protozoa, bacteria and fungi but a new born ruminant stomach is not fully developed as in adult. The stomach of newborn is quite similar to the mono-gastric stomach of non-ruminants. The newborn kid acquires this micro flora only after contact with previously faunated older animal. These microorganisms transferred from one to another animal by direct contact of saliva containing viable microbes (Becker and Hsiung, 1929). During rumination the microbes reach to the mouth of animal (Strelkov et al., 1933).

2.1.1 Bacteria

The stomach contains various types of bacteria immediately after birth. These bacteria are aerobic and facultative anaerobe (Fonty, 1984, Fonty et al., 1984, 1986). There are large number of amylolytic, proteolytic and lactate utilizing bacteria (10^8-10^9/ml) in calf in the first day of life and number increased as they mature (Anderson et al., 1987). The transfer of rumen bacteria occurs from animal previously containing bacteria to the bacteria free animal through direct contact (Kurihara et al., 1968). The strict anaerobic bacteria may appear about two days after birth. The metabolic group

of bacteria appears at very early age about after four days from birth in rumen of lambs as before consumption of solid feed and reached to the level of 10^6-10^8/ml (Fonty, 1984). At the age of 4 weeks, the predominating species were *Selenomonas ruminatium*, at 6-week age *S. ruminantium* was codominating with *Bacteroides ruminicola* and at the age of 8 weeks the bacterial flora became mixture of various species without any predominating species (Muller et al., 1984).

2.1.2 Protozoa

The faunation of rumen protozoa may take place when protozoa left in the food by one animal are consumed by another. The viable and active organisms that reach to host's mouth during rumination are killed by drying or exposed to air; hence the transfer is generally affected by mouth to mouth contact through saliva between the animal (Hobson, 1971). Faunation occurs in young ones, when their mother licks and grooms them and they swallow some saliva and digesta (Bryant et al., 1958, Fonty et al., 1984). After close contact with adult, protozoa have been detected in the stomach of calves two week after birth. This inoculation is frequent but protozoa do not immediately established because the rumen is too acid and protozoa cannot tolerate acidity (Purser and Moir, 1959). As animal start to consume forage, pH rises due to increasing salivary secretion, thus condition became favorable for establishment of ciliate protozoa population. When pH became 6.0, *Entodinia* became established (Bryant and Small, 1960) and at 6.5 the *Holotricha* and higher pH *Ophryoscolex* begin to appear. In lambs, ciliates could present at an early age of nine days mostly at 14 days and is rare at 21 days after birth (Eadie, 1962). In sheep the appearance of

ciliate begins at ninth day and completed on 21 day, *Entodinium* was the first genus to be appearing (Bryant and small, 1960, Eadie, 1967). Early weaning favors the establishment of ciliates (Naga and Elshazly 1968). Under routine feeding management the protozoa got re-establishment after 16 days following defaunation in sheep and goat. The *Entodinia* and *Holotricha* are the first to appear in rumen of goat and sheep (Santra and Karim, 1999). Thus protozoa population appeared in the order of *Eudiplodinium, Polyplastron* and *Eudiplodinum* appeared first in lambs, when they started normal feeding (Fonty et al., 1986). Establishment of genus *Diplodinium* is followed by *Polyplastron* in calves (Singh et al., 1988). *Entodinium sp.* is like *E. minimum*, *E. simplex* and *E. elongatum* establishment first and later on *E. caudatum*, *E. bursa*. In Anglo Nubian goats mainly *Entodinium sp.* was present in rumen from two weeks after birth to three months old (Olivera et al., 1989). *Holotricha* established at late stages but *Dasytricha ruminantium* established along with small *Entodinium sp.* (Groliecre et al., 1980). The late appearance of *Holotricha* is due to either unable to use carbohydrate or they might sited to the bottom of ventral sac of rumen, so they were not drawn in rumen sample or development of rumen is incomplete; hence the stomach conditions are not congenial to *Holotricha* or there were no inoculation source (Singh et al., 1988). In two groups of calves, a specific order of various species of protozoa establishment was observed that *Entodinium* appeared at 30 days, *Oscillospira* at 45 days, *E. bursa* at 60 days, *Polyplastron* at 75 days, *Diplodinium* at 90 days, *Dasytricha* at 135 days, *Isotricha* at 165 days and *Epidinium* at 180 days (Singh et al., 1988).

2.1.3 Fungi

The importance of anaerobic fungi in herbivore nutrition is still not well understood, but their undoubted ability to utilize major plant cell wall polysaccharides for growth and to produce a wide range of enzymes shows that they have the potential to contribute substantially to the plant fiber degradation in the alimentary tract of the host animal. The rumen fungi are establishment in rumen within first two week of age and reach the level of adult within 6 to 8 weeks of age (Orpin, 1989). Fonty et al., (1987) found that anaerobic fungi appeared in rumen of young lambs by 8 to 10 days after birth in the presence of adult animals, although their viability was not sure. They were found in all lambs until three weeks of age and then disappeared in 9 of the 11 lambs studied as soon as concentrate diet was given. The fungal population was mainly composed of *Neocallimastrix frontalis* and *Spheromonas communis* was found only sporadically. These microorganisms which had only previously been found in matured ruminants or when roughages rich diets are fed (Orpin, 1983/84) are apparently able to develop in the rumen before the ingestion of large amounts of solid feed. Anaerobic fungi could be isolated from esophageal preparations of saliva and from feces sheep. This indicated that mode of the transmission of the fungi could be direct oral contact and fecal contamination (Lowe et al., 1987a). However, as ruminants are not normally coprophagic, transfer of anaerobic fungi in feces would appear to be unlikely, although accidental contact wit feces particularly by contamination of the diet might occur. The anaerobic fungi of

feces might be disseminated from feces to herbage in nature, thus enabling transfer of anaerobic fungi between herbivores (Theodorou et al, 1994).

2.2 MICROBIAL POPULATION IN RUMEN

The microbial community is accommodated in the ecosystem of the foregut i.e. reticulo-rumen, which provide a highly specialized anaerobic environment. The rumen contains extremely diverse microbial community, which contains specially adapted bacteria, protozoa and fungi. The discovery of rumen protozoa in 1843 was the first identification of microorganisms in the ruminant stomach.

The bacteria are most abundant 10^{10} to 10^{11}/ml in the rumen (Hobson, 1988, Mackie et al., 1996). The rumen bacteria are divided into three general forms: Spherical or ellipsoidal called cocci; cylindrical or rod shaped called rods; and spiral shaped called spiral. Further, based on the morphological features of the character of cell surface, structures, rumen bacteria can be classified into 3 groups; gram positive, gram negative and mycoplasma. Besides, these bacteria are again classified into cellulolytic, proteolytic, amylolytic, lipolytic etc. on the basis of their nature of substrate utilization (Hungate, 1966).

Generally, not more than six different genera of ciliate are found in the rumen. The generic composition and overall size of the rumen ciliate population is determined by various interacting factors, important of which are types of host, its geographical location, the feed consumed and protozoa interspecies hostility.

The total number of protozoa varies approximately 10^5 to 10^6 cells/ml and majorities are of ciliates (Clark, 1977). The total number of ciliate protozoa varies with the diet of high digestibility, which provide readily available sources of nitrogen and energy, produce the largest ciliate population, although the frequency and amount of food consumed by the host, the diurnal cycle of the rumen, food particle size, salt concentration and dietary supplementation with antibiotics or other drug additives have all been shown to influence the protozoa population (Coleman, 1980).

The anaerobic fungi are unique among rumen microorganism in that they can penetrate the protein layer of plant fiber. The population of fungi is comparatively very less 10^3 to 10^5 zoospores per ml (Hobson, 1988, Mackie et al., 1996). The first rumen fungus isolated was poly-flagellated. Rumen fungi are categorized into two groups: Mono-centric and Polycentric. 3 genus viz.; *Neocallimastrix, Piromyces* and *Caecomyces* are mono-centric and 2 genus viz.; *Anaeromyces* and *Orpinomyces* are polycentric (Theodorou et al., 1996).

2.3 FACTORS AFFECTING BACTERIAL POPULATION

Investigations in the past by quite a few researchers have shown that there is a marked variation in the microbial population. The microbes inhabiting in the rumen varies and affected by various factors like, diet and its pattern, pH, season etc.

2.3.1 Diurnal changes

The concentration of total rumen bacteria decreased from 1 to 4 hours after feeding and increased slowly to a maximum between 12 to 20 hours in

sheep (Warner, 1966). The concentration of rumen bacteria in sheep fed to appetite in pens or pastures followed similar growth patterns to those in animals fed once daily. In cattle the total bacteria population was lowest at 1 hour after feeding and increased significantly between 1 and 2.5 hours after feeding as well as between 2.5 and 5 hour after feeding (Bryant and Robinson, 1968).

2.3.2 Diet

In general bacterial population is higher in those animals receiving high concentrate diet (Caldwell and Bryannt, 1966, Hungate, 1966, 1970, Dehority and Grubb, 1980, Leedle and Hespell, 1980). The bacterial numbers are equal or higher in animals fed on high roughage diets (Bryant and Robinson, 1968, Lantham, et al., 1971, Dehority and Grubb, 1977, Vander Linder, et al., 1984, Leedle et al., 1986). The inclusions of starchy concentrate in the diet increased the bacterial count (Punia, et al., 1981). Feeding the ionophores monensin or salinomycin was found to increase the percentage of resistant rumen bacteria (Dawson and Boling, 1983, Olumeyan et al., 1986). Some types of diets produce adverse effect on bacterial population. The carboxylic polyether ionophore antibiotics inhibit the gram positive bacteria with a corresponding enrichment of the gram negative population (Brulla and Bryant, 1980, Dennis et al., 1981). In ram, bacterial population decreased when fed on increased untreated whole cottonseed. (Sayed, et al., 2003). The legume based diet encouraged better rumen metabolites for growth and multiplication of rumen microbial population (Samanta et al., 2005).

2.3.3 Seasonal variations

The bacterial counts were lowest in winter and highest in summer (Hobson, et al., 1976). The bacterial concentration in animals during summer months (20.9×10^9/g) consuming high concentrate diet which decreased to less than 20 % of this value during winter (Orpin, et al., 1985). Giesecke and Vangylswyk (1975), measured viable bacterial count in rumen content from South Africa, viable counts taken during the dry season ranged from 13.36 $\times 10^8$/g in buffalo to 46.6×10^8 /g in springbok.

2.4 FACTORS AFFECTING PROTOZOAL POPULATION

2.4.1 Diet

Protozoa population in the rumen is exaggerated by varied nature of diet and diet pattern. The protozoa count increases after feeding (Bragg et al., 1986) and varies with the type of feed given. Concentrate produces greater effect on protozoa population as it increases with increase in concentrate in the diet in goats (Soni and Sharma, 1982). Santra and Karim (2001) observed higher protozoa population in *Kutchi* goat when fed on concentrate. Similar results were found by Sudha et al., (1996) in goats fed on 90% concentrate diet and sucrose infusions 10g/kg body weight twice a day. In *Barbari* goats the protozoa population was higher when fed on gram straw, berseem and pelleted concentrate feed in compare to those fed on only gram straw and berseem (Chaudhary and Singh, 2004). Protozoa population was found higher in Blue duiker fed on concentrate diet (Dehority, 1994). Similar results were found in male *Beetal* goats (Chaudhary and Ogra, 1997). Supplementation of vitamin in

diet affects protozoa population as in buffalo diet with niacin and nicotinic acid increases their population (Ghosh et al., 2003). Buffalo fed on *Subabool* diet contained higher protozoa population (Gill et al., 1992). The rumen degradation rate on microbial density in Taiwan native goats were affected by three different starch (maize) and two protein (soyabean), when these were fed on high starch and low protein degradability supported the highest number of *Holotrichs* whereas, rapid starch and protein degradability rate highest number of *Entodiniomorph* (Hung-Yenchi et al., 1995). The ciliate number (1.4) increased in Korean black goat when fed on diet having 15 g urea/day for 17 days. Similarly in swamp buffaloes diet with urea molasses mixture improves microbial population (Nguyen and Uden 2002). Protozoa count was greatest in animal given no or 100 % urea and least in those given 55 % urea nitrogen. This urea content also increases generation time of protozoa (Nour et al., 1979). In cows protozoa population increased with the diet consists of maize silage, Lucerne silage and meadow hay and concentrate mixture (Dolezal 2004). Likewise, barley in diet increased protozoa population (Nagaraja et al., 1995). Protozoa biomass was rapidly affected by dietary starch alone as increasing the dietary starch the protozoa biomass increased but further increased in starch caused protozoa biomass decline (Dikstra, 1994). The *Entodinia* population was higher in goats when fed on guinea and TSH grass (Misra et al., 1996). Total number of ciliates in rumen fluid rose with methionine hydroxyl analog supplementation in the diet of sheep (Vuyst et al., 1975).

It is found that in cattle calves bentonite supplementation diet increased protozoa population (Madhumohini, et al., 1997). Presence of some mineral elements affects the protozoa population as in bull their population found increased with supplementation of phosphorus with diet (Kocabatmaz et al., 1992).

The diet not only enhances the microbial population but also creates an adverse effect. Sheep, when fed on linoleic acid rich sunflower seed diet, protozoa number reduced (Ivan et al., 2003). The *Entodiniomorph* population reduced ($P<0.01$) by formaldehyde with diet in buffalo (Srivastava and Veeramani, 1993). Similar results were obtained by Broudiscou et al., (1994) that supplementation of linseed oil reduced protozoa number (from 565×10^3 to 191×10^3/ ml) Galindo et al., (2003) found that inclusions of *Brosimum allicastrum* and *Leucaena leucocephala* reduced the proteolytic and total viable microorganisms. When *Sesbania sesban* was fed to sheep, protozoa number decreased by 60% after four days but this population recovered after a further 10 days (Newbold et al., 1997). Alcohol in powdered form supplemented with diet decreased protozoa population (Chung and Lee, 1996).

2.4.2 Geographical factors

Abou Akkada and El shazly (1964), Naga et al., (1969) observed the protozoa population in both cow and buffalo calves and in sheep in from various regions in Egypt and suggested that the genus *Epidinium* is absent from all ruminants in Egyptian territory. There are geographical variations on the protozoa fauna of water buffalo from Brazil, Taiwan, Japan and Korea

(Williamms, 1986). *Holotrichs* are not detected in the animals of west Asia the variety of ciliates is larger in grazing than browsing animals in Europe (Giesecke, 1970) but there is greater variety of ciliate species in browsing animals in Africa. In Africa, *Dasytricha* and *Isotricha* spp. occur more regularly in browsers. The rumen ciliate fauna of Zebu cattle in Philippines resembled with the water buffalo of East Asia than Zebu cattle in India (Shimizu et al., 1983). Similarly cattle in Thailand and Philippines contained the same protozoa species as these found in India. The rumen microfauna of the family *Entodinia* from Turkish domestic sheep show more species diversity those Chinese, Japanese, Scottish, Canadian and Alaskan sheep (Oktem et al., 1997). Rastgeldi and Gocman 2003 found only two species of *Diplodinium* (*D. cristagali* and *D. flabellum*) first time in Turkish domestic goats.

2.3.3 Seasonal variation

There is little information on seasonal variation in microbial population because most of experiments are carried out in control conditions. Pearson (1965, 1969) observed a marked decrease in rumen protozoa concentration in mule deer from Utah and in white tailed deer from Texas during the winter months. Similar winter decreases in numbers occurred in red deer and sheep in Scottish Highland (Hobson et al., 1976). Westerling (1970) has reported a 45 % decrease in protozoa population in Finnish reindeer between August and November. The number of protozoa in cattle of Czechoslovakia ranged from 6.0×10^4 /ml to 10.0×10^4/ml in autumn and winter and from 10.0×10^4 /ml to

17.0×10^4 /ml of rumen fluid during spring and summer (Cruzha and Striz, 1977).

Protozoa population density of grazing sheep of South Africa varied in different seasons and mean counts were 100, 277, 455 and 278×10^3/ml in winter, spring, summer and April respectively (Vanderwath and Myburgh, 1941). Westerling (1970) found similar results for ciliate in Finnish reindeer and where the population was 3500×10^3 /ml in summer and 2000×10^3 /ml in winter. Protozoal concentration in Zebu cattle doubled between the dry (5.9×10^4 /ml) and wet (12.0×10^4 /ml) seasons in Senegal. Besides the total population seasonal effect had seen on differential population, during the wet season the genus Epidinium accounted for 6 % of the population and Holotrichs 7 % and Entodinium ranged from 35 % to 85 % with the remainder of the ciliates in the genus Diplodinium and in dry season Entodinium (89 %) with 5.7 % Diplodinium and 4.2 % Holotrichs (Bonhomme-Florentin et al., 1978).

2.3.4 pH

The optimum pH for microbial survival in rumen is 6.5 to 6.9 .in young ruminants the pH of stomach is too acidic and protozoa cannot tolerate it .As animal consumes forage the pH raises due to increased secretion of saliva. At pH 6.5, all protozoa established immediately (Eadie, 1962). Protozoa are quite sensitive to acidity with increase of pH, protozoa count increased (Purser and Moir, 1959) and cannot survive out side the range from 5.5 and 8.0 (Hungate, 1966). At very high acidities all protozoa are killed. In the defaunated animal the pH dropped steadily due to the accumulation of hydrogen ions (Chaudhary

et al., 1995). Rumen protozoa established in Anglo Nubian goats at pH 6.9 (Oliver, et al., 1989).

2.5 FACTORS AFFECTING THE FUNGAL POPULATION

Fungi, the imperative member of rumen ecosystem are also affected by type and nature of diet taken by host ruminant.

2.5.1 Diet

Bauchop (1979) indicated that diet had a substantial effect on fungal population, fibrous diet supplemented more than leafy diets. Highest numbers were found with chaffed Lucerne diet because of more stalky material than pelleted lucern diet. Fungal population were stimulated with the alfalfa diet to a much greater extent than that observed with coastal Bermuda grass, though NDF percent was more in case of coastal Bermuda grass (69.7 %) than alfalfa diet (32.8 %). Therefore, the general statement that most fibrous diets support the greater fungal population (Windham and Akin, 1984) as in cattle and buffaloes (Samanta and Walli, 1999, Samanta et al., 1999). Supplementation of maize strover with sulphate increases fungal population (Akin, et al. 1983). It was speculated that the presence of large amount of soluble carbohydrates in the rumen liquor not only inhibited the adhesion of cellulolytic bacteria to the feed particle (Fonty et al., 1987) but also hindered the germination of zoospores preferred to get attached to those feed particles which stayed longer in the rumen and were able to provide the full duration of life cycle i.e. about 24 hours (Grenet, et al., 1989). Forage hay or silage based diets stimulated the fungal growth in the rumen and increased their population (Grenet, et al., 1989b, Fonty

and Grenet, 1994). It is found that type of diet pattern also produce an effect on fungal population as in goat, higher fungal population was observed in block feeding in contrast to mash feeding (Samata et al., 2003). Rye grass at the leafy stage was an unfavorable feed item for the fungi. This feed item did not cause any hinderness for the establishment of fungi in the rumen but reduced their development (Grenet, et al., 1989a). The use of very young grass (rich in nitrogen, soluble carbohydrates and highly digestible) allowed extensive fermentations in the rumen and gave a higher level of volatile fatty acids. In addition to this, due to little lignifications and lesser retention time in rumen, younger grass little scope to fungi for attachment and subsequent development. Concentrate and soluble sugars, fat or oils in diet reduces fungal population (Fonty and Grenet, 1994). In the same way sodium chloride treated rice straw and concentrate suppress the fungal population in sheep (Cann et al., 1993).

2.5.2 Time

Fungal zoospores were found to get attached to rice straw fragments 15 minutes after rumen incubation and 30 minutes many germinated (Ho, et al., 1991). Similar rapid attachment of zoospores to plant fragments in the rumen was reported by Bauchop and Mountfort (1981) and Ho, et al., (1988b). Sporangia of the fungi were small, mostly spherical or ovoid, at 24 hours, fungal colonization of the straw fragments were very profused and dense network of hyphae were observed in the sclerenchyma and vascular tissues by. By 48 hours, much of the softer thin walled tissues of the rice straw fragments were degraded and remaining sclerenchyma and vascular tissues were

extensively colonized by fungi. In place of large spherical and ovoid sporangia a large number of ovoid structures having zoospores or young sporangia, were present both out side and within the cells (Ho, et al., 1991).

Roger et al., (1993) observed that after 15 hours of incubation with *Neocallimastrix frontalis* the tissues of straw and maize were almost completely covered by an abundant mycelium. After 24 hours the inner part of the parenchyma was degraded and after 48 hours it was separated from the stem. At 72 hours of incubation the degradable tissues (phloem and parenchyma) disappeared.

2.5.3 Feed frequency

Beet diets (comprised of 8.2 kg capax fodder beet and 7.4 kg Lucerne hay) offered in single meal did not cause fungal colonization on substrate suspended through nylon bag. The number of fungi was also low in rumen fluid. But during offering the same beef diet in six different meals, fungi attached to the substrates and number of fungi in rumen fluid was also more (Grenet et al., 1989a).

2.5.4 Species of fungus

Members of the genus *Caecomyces* seemed to be least capable of penetrating plant fibre among all the anaerobic fungi (Gordon and Phillips, 1989, Roger et al., 1992). This was due to the presence of spherical holdfast unlike mycelial rhizoids of *Neocallimastrix, Orpinomyces, Anaeromyced and Piromyces species*. Thus *Caecomyces* were unable to penetrate deeply into plant tissues (Fonty and Joblin 1990).

2.6 INTERACTION AMONG MICROBES

The life of ruminant is led in mutual relation with microbe i.e. anaerobic bacteria, fungi and protozoa. In rumen ecosystem both synergistic and competitive microbial interactions take place. These microbes interact each other. The presence of protozoa induces the rod forms of bacteria whereas streptococcus increased in ciliate free lamb (Maugaret and Gill, 1971). The presence of protozoa reduced the fungal carboxymethyl cellulase activity to about half of that in pure culture of fungi (Widyastuti et al., 1995). The protozoa affect the microbial population in cattle as it reduces the number of total cultural anaerobic bacteria, amylolytic bacteria and cellulolytic bacteria where as it increased methanogenic, sulphate reducing bacteria and anaerobic fungi (Arakaki et al., 1994). The investigation of Stewart and Richrdson (1989) indicated that coculture of rumen fungi and methanogenic bacteria enhanced the resistance of fungi to growth promoter antibiotics for animals. The number of fungi, total viable or cellulolytic bacteria was lower after inoculation with protozoa in defaunated rumen (Mathieu et al., 1996). The numbers of bacteria in defaunated animals are greater than in normal faunated animal (Bryant and Small, 1960). It is due to the predation of bacteria by porotozoa. *Ophryoscolex caudatum* could obtain the amino acids for growth by engulfment of rumen bacteria (Coleman and Reynold, 1982). Rumen protozoa are responsible for hydrogen production that is used in methane formation by bacteria (Prins and Von Hoven, 1977). Thus it is clear that rumen bacteria interact with methane bacteria. Protozoa are directly involved in stimulating the activity of cellulolytic

bacteria (Jounay and Martin, 1997). Presence of fungal population affects, the cellulolytic (2.5 fold), hemicellulolytic (2.9 fold) and total bacterial count (2.5 fold) and fungal count (4.4fold) increased by administration of *Piromyces* fungus in blue bull (Paul et al., 2004). Rumen fungi interact with a varity of protozoal and bacterial species in their ecosystem. Bauchop and Mountfort (1981) showed a synergistic influence of methanogenic rumen bacteria on cellulolytic activity of fungi increased to an extend of digestion by 55% and altering the fermentative products towards higher amounts of acetate, methane and carbon dioxide at the expense of formate and hydrogen. Coculture of rumen fungi and methanogenic bacteria enhanced the resistance of fungi to growth promoting antibiotics i.e. monensin and lasalocid for animals. Defaunation increased fungal zoospores as these are predated by rumen protozoa (Orpin, 1981).

2.7 ROLE OF RUMEN MICROBES

The microbial ecology of rumen is very complex. Different species of bacteria, protozoa and fungi are involved in digestion of plant material in ruminants. The microbial population present in rumen provides ability to the ruminants to utilize the feed material like lignocellulosic agricultural byproducts, non-protein nitrogen etc. These microbes also hydrolyze some toxic principles present in the feed taken by the animals making them resistant to those toxic. The rumen microbes trapped the energy hidden in the cellulose in roughage feed. The measurement of various parameters in faunated, defaunated, animals that ruminal environment can be markedly altered by the presence of

protozoa (Williamms and Coleman, 1988). Inspite of complicated inter relationship among the various groups of microorganism in rumen ecosystem, bacteria are believed to play a major role because of their numerical predominance and metabolic diversity (Raizada et al., 2003).

2.7.1 Metabolism of cellulose and hemicellulose

Cellulosic roughage constitutes the major part of ruminal diet. Their fermentation is mediated through microbial ecosystem in rumen. The cellulase enzyme systems of bacteria, fungi and protozoa are quite different (Matsui et al., 1992). The rumen fungi convert cellulose into acetate, formate, lactate, malate, ethanol, succinate and hydrogen (Joblin and Naylor, 1993). Similarly in sheep, fungi have the ability of degrading dry matter and fibre from different types of feed (Ghoorchi et al., 2003). The bacteria attach to the cellulose particle through glycocalyx (Agarwal, 1999). These bacteria attack on cellulose and convert them into energy yielding substrates like volatile fatty acids. Some bacteria like *Ruminoccus albus, Fibrobacter succinogens, Clostridium lochheasii* and *Eubacterium* are cellulose degrading bacteria (Kamra, 1999).

2.7.2 Metabolism of starch

Rumen protozoa of the genera *Oligotrich* and *Holotrichs* have strong alpha amylase activity. Oligotrichs liberate maltose from starch. *Entodinium caudatum, Epidinium caudatum, Polyplastron multivasiculatum, Isotricha species* and *Dasytrica ruminatium* are all starch digesters (Abou Akkada and Howard, 1960). 84 % of the starch of a ration containing 47 % ground shelled maize was digested in the rumen in the presence of *Entodinium* and

Diplodinium spp, Epidinium caudatum and *Eudiplodinium magii* possessed the highest concentrations of amylase (Coleman, 1986). Starch digestion by bacterial amylase takes place through glycocalyx (Cheng et al., 1990). The pH optimum for amylase enzyme is 6.0 (Agarwal et al., 1991). *Streptococus bovis, Ruminobacter amylophilus, Ruminobacter ruminocola* and *Succinimonas amylolytica* are starch-degrading bacteria. Many anaerobic fungi can utilize and grow over starch as a sole source of energy (McAllister et al, 1993) Ruminal fungus *Neocallimastrix* produces alpha amylase and its products were mainly maltriose, maltotetraose and longer chain Oligosaccharides (Douglas et al., 1988).

2.7.3 Metabolism of protein

Protein eaten by the host ruminant passes into the rumen where it is degraded by the microorganisms. Rumen protozoa have exopeptidase activity (Nagasawa, et al., 1992). The relative importance of the protozoa in the rumen depends on its number, the rate at which they synthesize protein and finally on the rate at which the protozoa leave the rumen. Species of *Entodinium, Diplodinium* and *Ophryoscolex* have been also shown to be proteolytic (Hungate, 1966). The higher concentration of ammonia nitrogen, TCA precipitable protein, soluble nitrogen, urease activity, plasma protein and urea were significantly lower in defaunated cross bred calves fed on normal or no protein diet (Pal et al., 1998). Most of the species of rumen bacteria are proteolytic but *Ruminobacter amylophilus, Butyriivibrio fibrisolvens* and *Prevotella ruminocola* are major proteolytic bacteria (Agarwal, 1999). Wallace

et al., (1996) reported only common bacteria species *Prevotella ruminocola* possessing high dipeptidyl peptidase activity. Anaerobic rumen fungi *Neocallimastrix frontalis, Piromonas sp.* and *Orpinomyces joyonii* have proteolytic activity (Asao et al., 1993). Rumen fungi have amino peptidase activity (Michel, et al., 1993).

2.7.4 Predation of bacteria

Eadie and Hobson, (1962) first showed that when lambs (fed on hay and concentrates) had been kept ciliate free from birth were inoculated with rumen ciliates, the number of small bacteria declined from 36×10^9 to 14×10^9 /ml. The numbers of bacteria in defaunated animals are greater than in normal faunated (Eadie, 1962). It is due to consumption of bacteria by protozoa for food. Protozoa non-selectively engulf small bacteria and that the decrease in bacterial volume. On defaunation the number of free, but not particle associated bacteria attached to starch grains reduced (Kurihara et al., 1978).

2.8 EFFECT OF DEFAUNATION ON RUMEN METABOLITES

The presence of protozoa in the rumen has been shown to influence the volume of the digesta, the concentration and population of VFAs, the levels of NH_3N, pH etc. changes in these parameters will influence ruminal function with resultant beneficial or detrimental consequences for the host. The presence or absence of protozoa in rumen has been observed to have an impact on their host. This produces direct or indirect effect on host physiology (Kamra et al., 2000). The physical characteristics of the rumen environment are changed on defaunation.

2.8.1 Change in pH

The rumen pH value ranges from 6.5 to 6.8. Defaunation have an extent effect on rumen pH as it usually decreased on defaunation (Chaudhary 2002). Hydrogen ions are accumulated on defaunation causing low pH in rumen (Chaudhary et al., 1995). There was no effect of defaunation on *Murrah* buffalo calves fed on wheat straw and concentrate mixture (Chaudhary, et al., 1995).

2.8.2 Bacterial and fungal population

On defaunation the bacterial counts increased as protozoa engulf bacteria and similar effect on fungi. The numbers of bacteria in defaunated animals are greater than in normal faunated animal (Bryant and Small, 1960).

2.8.3 Rumen volume and rumen flow

The rumen volume and retention time have been reported to be either decreased or increased or no change following defaunation (Chaudhary 2002). Dafaunation refers to higher duodenal microbial nitrogen flow (Eugene et al., 2004).

2.8.4 Total concentration and molar proportion of VFA

The defaunation of rumen is associated with decrease in total VFA concentration. Total VFA concentration was higher ($P<0.05$) in faunated *Murrah* buffaloes (Chaudhary et al., 1995). The inoculation of single protozoa species or genus (*Polyplastron multivesiculatum* or *Entodinium* sp) to Mexoxenic lamb resulted in an increase in the ruminal VFA concentration and change in the molar proportion of the principal acids acetate, propionate and butyrate (Fonty et al., 1983). The molar proportion of acetate was lower in

faunated than unfaunated group but the proportion of butyrate and propionate was lower in unfaunated group (Nangia and Sharma, 1994). As the protozoa concentration increased in rumen, the concentration of acetate, butyrate and iso-acids increased (Mathieu et al., 1996). In the absence of protozoa in rumen, the acetate – propionate ratio and butyrate proportion of VFA were reduced in calves (Schonhusen et al., 2003). The concentration of VFA was higher in faunated than unfaunated goats (Itabshi and Kandatsu 1975).

2.8.5 Ammonia Nitrogen (NH_3-N)

Defaunation results decrease in ammonia levels (Nhan et al., 2001). The concentration of ammonia nitrogen in SRL was found low with the lower population of ciliate (Ivan et al., 2003). Concentration of NH_3-N in rumen was 100 % higher in faunated than unfaunated goat and cattle (Itabshi and Matsukawa, 1979). Similar results were obtained by Mathieu et al., (1996) in sheep. In *Murrah* buffalo calves, the NH_3-N was higher in defaunated than refaunated calves (Chaudhary, et al., 1995).

2.8.6 Methane production

Methane production was reduced by defaunation (Kreruzer et al., 1986, Santra et al., 1994). Methanogenesis is an energetically wasteful process and its suppression may be beneficial (Ushida and Jouany, 1996, Chandramani et al., 2001) for protecting the earth from the detrimental affects of those greenhouse gases.

2.8.7 Nutrient utilization

Digestion of all fibre components in the rumen environment changed on defaunation (Ushida and Jouany, 1990). The rumen fermentation of starch and cell wall decreased on defaunation (Jouany and Ushida, 1999). It reduced the digestion of structural carbohydrates in buffalo calves (Chaudhary et al., 1995). On defaunation DM intake increased from 60.20 to 64.08 in murrah buffalo (Chaudhary et al., 1995). The DMI, metabolic energy intake, were higher in defaunated than faunated heifers (Gupta et al., 1983, Demeyer et al, 1986 Krinshna Prasad et al., 2000). Defaunation increased digestibility of DM, OM, CP, and ADF (Kreuzer et al., 1986, Krishna Prasad et al 2000). Defaunation also reduced the digestibility of dry matter, organic matter, NDF, ADF, cellulose, hemicellulose ($P< 0.01$) in calves (Chaudhary and Srivastava, 1995).

2.8.8 Body weight and wool gain

The changes in the rumen function associated with defaunation suggest that it might have an effect on animal productivity. The negative or no responses of defaunation were observed in matured animals that do not respond to increased nitrogen availability to lower tract. The higher wool growth is reported in defaunated sheep. Wool growth is dependent on the amount of protein reaching in the lower tract particularly sulphur containing amino acids and higher flow of these amino acids to lower tract were reported in defaunated animals (Chaudhary, 2002).

2.8.9 Some other effects

Defaunation increased the total nitrogen and TCA nitrogen concentration in *Murrah* buffaloes (Chaudhary et al., 1995). More protein will be available for intestinal digestion by the host animal (Bird, 1991).

Chapter 3

Material and methods

3. MATERIALS AND METHODOLOGY

Ruminants mammals like goat depend for their survival on classic symbiotic association with microorganisms of the alimentary canal. The diet of grazing and browsing goats consists largely of plants structural carbohydrates like cellulose, hemicellulose and proteins. The goats themselves are unable to digest these plants biomolecules. The symbiotic microorganisms (bacteri, protozoa and fungi) of the alimentary tract hydrolyze these plant complex biomolecules under strictly anaerobic conditions (Hungate, 1966, Czerkawski, 1986) with the production of volatile fatty acids, microbial protein, vitamin etc. In order to curtail the cost of starter ration or follow the early weaning, for economic livestock production, it is utmost important to know the earliest date of establishment of different microbial community in newborn kid. To understand and know the role of each group of microorganisms, antibiotic should be charged against specific group under particular dietary regime. The microbial population of the rumen is diverse and before 1975, it was thought to be consists principally anaerobic bacteria and protozoa. The genius work of Orpin (1975) challenged the earlier hypothesis that the fungus could not survive anaerobically and was able to isolate the anaerobic fungus from ovine rumen, lacking mitochondria but having hydrogenosome for respiration. Later on, the anaerobic fungus was reported in various spp. like cattle (Bauchop, 1979), buffalo (Samanta et al., 1998), etc. Under these circumstances, attempts were made to study rumen function of local goat with special reference to type of anaerobic fungus. Rumen studies involve several scientific areas including

anaerobic microbiology, biochemistry, nutrition and animal management. Thus an endeavor is made to out line the materials and methods to conduct the studies on establishment of rumen microorganisms in kid as well as to define the role of different groups of microbes on nutrient utilization in adult goat.

The present investigation was conducted into two phases at the central experimental farm of Indian Grassland and Fodder Research Institute, Jhansi.

3.1 ESTABLISHMENT OF RUMEN MICROBES IN FOREGUT OF KID

To know the date of establishment of different microbial communities in the foregut six newborn male kids of local breed (Bundekkhand region) were procured from the Central Experimental Farm. The average body weights of newborn kids were 1.9 to 2.0 kg. The local breed were characterized by completely black in colour, medium body size, roman nose etc.

3.1.1 Housing of kid:

The kids were kept with their dam in well ventilated shed with the free access to water.

3.1.2 Feeding of animals:

The dams of the kids were offered *ad libitum* dried grass + 300 - 350 gram concentrate mixture. The newborn kids were initially permitted to suck dams milk (birth to 3rd day) but latter on (4th day onwards) sucking was stopped. Instead dams were milked thrice in a day and offered to kid @ 1/10th of body weight till the age of 4th week. Besides dried stylo leaves were given for *ad libitum* nibbling. From 5th week onwards dietary regime comprised of milk @

1/12th of body weight, *ad libitum* stylo leaves along with concentrate (50 g/daily) till the age of eight week.

3.1.3 Collection of foregut content:

Stomach content was drawn on alternate days from the each kid from first day of birth till the fully functional rumen i.e. about two months of age. The stomach content was collected by Ryle's tube and kept in pre-gassed 125 ml medicinal vial for studying the biochemical attributes as well as to know the establishment of rumen microbes.

3.1.4 Analysis of biochemical parameters of kid's foregut

The foregut's contents were looked for analysis of pH, ammonia nitrogen and total volatile fatty acids. These parameters are indicative of rumen development as the survivability of microbes depends upon the conducive environment enabled by those attributes.

3.1.4.1 pH:

The pH of the stomach contents was recorded immediately after collection using ADCO digital pH meter, which was first standardized by standard buffer solution.

3.1.4.2 Ammonia nitrogen (NH_3-N):

Ammonia nitrogen in the stomach contents was estimated by following the method of Conway (1962). 1ml of 4% boric acid having mixed indicator was put in the inner chamber of Conway dish. 1 ml of sample was taken at one side of dish in outer chamber and 1ml saturated sodium carbonate solution was poured at another side of outer chamber. These Conway dishes were covered by

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greased plate to make them airtight and slightly tilted and kept for about four hours at 37 ^{0}C the inner boric acid was titrated against 0.01 N H_2SO_4 and the amount of NH_3-N was calculated.

Calculation:

$$NH_3\text{-N (mg/1000 ml SRL)} = \frac{\text{Titer value} \times \text{Normality of } H_2SO_4 \times 0.14 \times 100}{\text{Volume of aliquote (ml)}}$$

3.1.4.3 Total Volatile Fatty acid (TVFA):

The total volatile fatty acids concentration was determined by the method of Bernett and Reid (1956). 2 ml of sample was taken in Markham's distillation apparatus. The sample was steam distilled in the presence of 2 ml of oxalate buffer. About 100 ml of distillate was collected under the cold jacket and then titrated with 0.01 N sodium hydroxide solution. Phenolphthalein was used as indicator, which gave light pink colour at the end point.

Calculation :

$$\text{TVFA (mM/100 ml SRL)} = \frac{\text{Volume of 0.01 NaOH} \times \text{ normality of NaOH} \times 100}{\text{Volume of sample taken}}$$

3.1.5 Enumeration of bacteria:

Total viable bacteria were enumerated in the foregut of kid by following the method of Hungate (1966). The carbon dioxide gas was first passed through heated copper column of Gassing Manifold (Biosystem) to make the gas free from oxygen. Copper reacts with oxygen and transformed into cupric oxide. Hence the gassing manifold system supplies the oxygen free carbon dioxide during the culturing of anaerobic microorganisms. The freshly collected

samples from kid's foregut or adult goat were transferred to the laboratory for enumerating the bacteria population. The serial dilution was made in anaerobic diluents up to the level of 10^8 and charged the roll tubes with the diluted inoculums. After inoculation, the incubation was carried in BOD incubator at 39 ± 1^0 C. The bacterial colonies were counted after 48 hours of incubation and bacterial population was enumerated by multiplying with dilution factors.

3.1.5 Anaerobic media for enumeration of total viable bacteria

The composition of the media (Joblin, 1981) is given in table 3.1. It is comprised of salt solutions A and salt solution B, glucose and cellobiose as energy source, sodium bicarbonate and cysteine hydrochloride as reducing agents and resazurin as indicator.

Table: 3.1 Media for enumeration of total viable bacteria (Joblin, 1981)

INGREDIENTS	QUANTITY
Salt solution A	17 ml
Salt solution B	17 ml
Clarified rumen liquor	15 ml
Yeast extract	50 mg
Tryptopan	100 mg
Glucose	100 mg
Cellobiose	100 mg
0.1% Resazurin solution	0.3 ml
0.05% Haemin	0.2 mg
5% Sodium bicarbonate	5.0 mg
5% Cystein hydrichloride	2.0 mg
Distelled water	43.6 ml
Agar	2.0 g

The composition of salt solution A and salt solution B is presented in table 3.2 and table 3.3 (Bauchop and Mountfort, 1981).

Table: 3.2 Composition of salt solution A (Bauchop and Mountfort, 1981)

SALT	QUANTITY (wt/vol)
KH_2PO_4	0.3 %
NaCl	0.6 %
$(NH_4)_2SO_4$	0.3 %
$CaCl_2$	0.03 %
$MgSO_4$	0.03 %

Table: 3.3 Composition of salt solution B (Bauchop and Mountfort, 1981)

SALT	QUANTITY (wt/vol)
KH_2PO_4	0.03 %

The composition of anaerobic diluents (Bryant and Burkey, 1953) is presented in table 3.4.

Table 3.4 Composition of anaerobic diluents (Bryant and Burkey, 1953)

INGREDIENTS	QUANTITY
Salt solution A	5.0 ml
Salt solution B	5.0 ml
5% cystein hydrochloride	4.0 ml
5% sodium carbonate	10.0 ml
0.1% Resazurin	0.2 ml
Distilled water	175.8 ml

Clarified rumen fluid (CRF) for the media:

The freshly collected rumen fluid was stained through double layer of muslin cloth and clarified through centrifugation @ 12,000 rpm for 30 minutes and kept at 5^0C. The rumen liquor was thawed before adding the media. This clarified rumen fluid was used to provide unidentified growth factor to the anaerobic fungus.

0.05% Haemin solution:

0.05g haemin powder was dissolved in a solution of 1:1 ethanol and 0.05 M sodium hydroxide solution.

Anaerobic media preparation:

All the quantified ingredients except sodium bicarbonate and cysteine hydrochloride (reducing agents) were taken in 250 ml flat bottom flask. The pH was adjusted with the range 6.6 to 6.8. The medium was boiled to free it from dissolved oxygen and cooled under the flow of carbon supplied through Biosystem gassing manifold. The reducing agents were added in the media before its solidification under continuous flow of carbon dioxide. The flask containing media was closed by rubber cork and tied through wire and autoclaved. The colorless or slight yellow appearance of media was identification of perfectly anaerobic condition while any pinkish appearance was identification of aerobic or imperfect anaerobic condition.

3.1.6 Enumeration of protozoa:

The freshly collected stomach contents were brought to the laboratory at a temperature between 35- 40^0C while maintaining all anaerobic conditions.

3.1.6.1 Fixing

The 2 ml of rumen liquor was mixed with 2 ml of 50 % formalin solution and kept at room temperature. One or two drops of methyl green dye were added to stain the nucleus.

3.1.6.2 Microscopic count of protozoa:

The number of protozoa was counted in a known volume of medium. The total number as well as differential numbers was calculated as described by Kamra et al. (1991)

Protocol:

1. The stomach contents of either kid or adult goat collected were transferred to the laboratory by maintaining anaerobic conditions during transport.
2. Mixing of rumen liquor with preservative.
3. Two drops of preserved sample were dropped on the two cavities of haemocytometer.
4. After putting cover slip, 30 microscopic fields were counted for protozoa.
5 Average numbers of protozoa were calculated per microscopic field.
6. The number of protozoa was calculated as below:

$$N = \frac{n.\ A.\ D}{a.\ v.}$$

Where, N= Number of protozoa /ml of rumen liquor

n = Average number of cells /microscopic field

A = Area of slides on which the diluted sample of rumen liquor is spread (area of the cavity of haemocytometer)

D = Dilution of rumen liquor

a = Area of microscopic field

V= Volume of diluted rumen liquor in the cavity.

3.1.6.3 Identification of protozoa:

Protozoa preserved in formalin solution were stained by Lugol's iodine and allowed to stand for about 20 minutes. Few drops of stained rumen liquor sample were examined under microscope; skeletal plates stained deep brown.

3.1.7 Enumeration of fungi:

Fungal enumeration and counting was conducted by following the Hungate's roll tube technique (Hungate, 1966) as adopted by Samanta and Walli (1999). The composition of the anaerobic media (Joblin, 1981) for enumeration of anaerobic fungus is presented in table 3.5. It contained salt solutions A and B (Table 3.2 and Table 3.3), clarified rumen fluid, cellobiose as energy source, sodium bicarbonate and cysteine hydrochloride as reducing agents and resazurin as indicator.

Table: 3.5 Anaerobic media for enumeration of fungus (Joblin, 1981)

INGREDIENTS	QUANTITY
Salt solution A	17 ml
Salt solution B	17 ml
Clarified rumen liquor	15 ml
Yeast extract	50 mg
Tryptopan	100 mg
Cellobiose	200 mg
0.1% Resazurin solution	0.3 ml
0.05% Haemin	0.2 mg
5% Sodium bicarbonate	5.0 mg
5% Cystein hydrichloride	2.0 mg
Distelled water	43.6 ml
Agar	2.0 g

3.1.7.1 Antibiotic stock solution

A. Penicillin stock solution;

The stock solution for sodium salt of benzyl penicillin was prepared, having a concentration of 2×10^4 IU per ml and sterilized through membrane filtration (0.22 µm pore diameter). This was gassed through oxygen free carbon dioxide and 0.25 ml was added in each roll tube.

B. Streptomycin surface stock solution

Streptomycin sulphate stock solution was prepared at a concentration of 2 mg per ml and sterilized through membrane filter (0.22 µm pore diameter). This was gassed through oxygen free carbon dioxide and 0.25 ml was added in each roll tube.

3.1.7.2 Processing of rumen liquor:

Freshly collected rumen liquor from goat foregut was used without straining as a source of inoculums and stirred for 5 to 7 minutes on a magnetic stirrer. The stirring was performed under the continuous flow of carbon dioxide in front of flame. Then 1 ml of stirred rumen liquor was dispensed in 20 ml glass vial containing 9 ml of anaerobic diluents to get required dilution. This gave a dilution of 1:10 (10^2)

3.1.7.3 Dispensing of anaerobic media:

The sterile roll tubes were gassed with oxygen free carbon dioxide for 2 minutes under aseptic condition. Then the molten media (4.0 ml) was dispensed in each tube and kept in the water bath at 45^0C.

3.1.7.4 Inoculation into roll tubes:

The inoculums (0.5 ml) of desired level of dilution added in roll tubes containing molten media and antibiotic solutions. The tubes were then rolled over platform of crushed ice to solidify the media uniformly as a thin film on the inner walls of the roll tube. The tubes were kept in incubator at a temperature of 39 $\pm 1^0$C. During the incubation the roll tubes were regularly inspected for appearance of colonies visible through naked eyes. The number of colonies was then counted.

3.1.7.5 Isolation and maintenance of fungal isolate:

The anaerobic fungal colonies, which appeared in roll tubes, were transferred to a media containing 0.3 % soft agar media in the presence of recommended dose of antibiotics.

3.1.7.6 Identification:

Identification was done up to genus level based on morphological features of zoospores, sporangia and nature of rhizomycilia (Trinci et al., 1994, Theodorou et al., 1996, Samanta et al., 1998) under the phase contrast microscope.

3.1.7.7 Analysis of metabolic product of anaerobic fungus

The potential isolates were grown in sealed medicinal vial containing 15 ml 0.35 % agar containing cellobiose as energy source. After 5 days of inoculation supernatant was collected by centrifuging at a speed of 10,000 rpm. Thereafter the supernatant was used for various fungal metabolites and enzyme activities.

Lactic acid:

It was determined according to the method described by Barker and Summerson (1941). A fixed volume (0.3 ml) supernatant was taken in test tubes and 0.7 ml distilled water was added to make 1 ml. Then 6 ml H_2SO_4 (containing 8.33 ml 4 % Cu SO_4.5 H2O) was added and shaken. Boiled in hot water for 5 minutes and cooled. The added 0.1 ml para-hydroxy phenyl (1.5 % in 0.5 % NaOH solution). Shake well and waited for 3o minutes in 30^0C water bath. Again boiled for 90 seconds in water bath and cooled. OD was recorded at 570 nm.

Fractional Volatile fatty acids (FVFA):

The fungal isolates were grown in 4 ml of cellobiose soft agar media. After five days of growth at 39 ± 1^0 C, the culture supernatant was harvested by centrifuging at 10000 rpm. Then 1 ml of supernatant was mixed with 4 ml of 20 % Meta phosphoric acid (prepared in 5 N H_2SO_4). These were kept for overnight at room temperature and centrifuged at 4000 rpm for 20 minutes (Erwin et al., 1961). The supernatant was used for fraction of VFA on Gas Liquid Chromatography fitted with flame ionization detectactor. Standard VFA mixture containing acetate, propionate and butyrate were injected to GLC before loading the sample. 2µl of centrifuged sample was injected in gas liquid chromatograph.

Calculation:

Area of peak = 1/2 height × width of the base

Area of peak for acetate, propionate and butyrate were calculated and compared with the area of peaks of standard and presented accordingly.

3.1.7.8 Determination of fibrolytic enzyme secreted by anaerobic fungus:

The different fungal isolate showing vigorous growth in laboratory were grown in cellobiose soft agar in medicinal vials capped by rubber cork and sealed by aluminuium seals. These were incubated at 39 ±1 ^{0}C for a period of five days after which the supernatant containing enzymes were harvested by centrifugation at a speed of 10,000 rpm for 15 minutes. The supernatant obtained was used for endoglucanase, xylanase, Fp-ase (filter paper ase).

Endoglucanase activity:

The method of Mountfort and Asher (1988) was followed for estimation of endoglucanase activity. The total assay system (2 ml) contains 1 ml substrate (1 %) CMC dissolved in 0.1 M citrate phosphate buffer, pH 6.50, appropriate volume of enzyme and 0.1 M citrate phosphate buffer (pH 6.5). After 2 hours of incubation at 50^{0}C in water bath, the reducing sugar released from carboxy-methyle cellulose is estimated by the method of Nelson, modified by Somogyi (1952).

FP ase activity:

In total assay system of 2 ml contain 13.4 mg of Whatman's filter paper disc (10 discs), citrate phosphate buffer (pH 6.5), and appropriate volume of enzyme. After 2 hours of incubation at 50^0 C in water bath, reducing sugar was determined as per the method of Somogii (1952).

Enzyme unit (IU):

It is defined as the micromole of glucose released per minute. Specific activity is defined as units (IU) of activity per mg of protein.

Xylanase:

About 1 % (wt/vol) of the larch wood xylan was used as substrate. In the assay system of 2 ml volume contain 1 ml 1 % xylan, dissolved in 0.1 M citrate phosphate buffer. Reaction is carried out at 50^0C water bath for a period of 1 hour. Reducing sugars were estimated by the method of Somogyi (1952).

Enzyme unit (IU):

It is defined as micromole of reducing sugar released per minute. The specific activity is expressed as units (IU) of activity per mg of protein.

3.2 ROLE OF MICROBES IN RUMEN FERMENTATION:

To know the fermentative role of different groups of microbes, the experiment (Phase II) was conducted at central experimental farm.

3.2.1 Animals:

12 adult local male goats were divided into four groups comprising of three in each group (Table 3.6).

Table 3.6 Experimental schedule of animals:

GROUP	TYPE
T_1	Control
T_2	Defaunated
T_3	Fungus free
T_4	Bacteria free

3.2.2 Housing of animals:

The experimental goats were kept in four separate, well ventilated cages with free access to water and feed. Strictly separate utensils were used for each group to avoid any contamination.

3.2.3 Feed of animals:

All the treatment groups were fed on the diet containing concentrate and roughage (dried chaffed grass and *Leucaena* leaves at 50:50). Each group was offered 2.0 kg feed per day consisting of 800 g concentrate and 1200 g roughage i.e. mixture of grass and *Leucaena* leaves.

3.2.4 Dose of antimicrobial agents:

Table : 3.7 Dose of antimicrobial agents

Type	Dose of antimicrobial agent
T1	No dose (Control)
T2	8.0 g SDS /100 kg b.wt
T3	200 mg/day Fluconazole
T4	4.0 g/100 b.wt. (2 tablets of 500 mg of tetracycline group)

T_1 Group:

T_1 group was controlled having normal micro flora in their gastrointestinal tract. No antimicrobial treatment was given to this group.

T_2 Group:

This group was defaunated. Goats were protozoa free, but having bacteria and fungi in their gastrointestinal tract. This group was defaunated to known the role of protozoa on rumen function. The goats were defaunated chemically by Sodium lauryl sulfate @ 8.0 g /100 kg body weight (Santra et al., 1994). The average body weight was 25 kg, thus 6.0 g of SDS (2 g SDS /goat) was dissolved in 30 ml of distilled water. 10 ml of this SDS solution was given orally to each goat. This treatment group was maintained defaunated till the completion of the experiment.

T_3 group:

This group was fungi free. Each goat of this group was charged with Fluconazole capsule (Trade name Forcan) @ 200 mg /day for 7 days. Following dosing for one week, the rumen liquor was collected for the presence of anaerobic fungus and it was found fungus free at rumen levels. Therefore, the fermentation of this group of goats was carried by only bacteria and protozoa.

T_4 Group:

This group was bacteria free and treated with antibacterial agents @ 4.0 g/ 100 kg b. wt. The average body weight of each animal of this group was 23 kg. Each goat was given 2 tablets (500 mg) of tetracycline. Therefore, the rumen fermentation was carried out by protozoa and fungi.

3.2.5 Collection of rumen liquor:

Rumen liquor was collected by stomach tube before offering of feed in the morning. The samples were transferred to the laboratory at a 39^0 C under

strictly anaerobic conditions. This rumen liquor was processed for microbial population and various rumen metabolites.

3.2.6 Analysis of rumen metabolites:

The various rumen biochemical parameters like pH, ammonia nitrogen and total volatile fatty acids were determined as described earlier. Total nitrogen, TCA precipitable nitrogen, non-protein nitrogen were determined as below.

Total Nitrogen:

Rumen liquor was analyzed for total nitrogen by the standard Microkjeldahl method. 10 ml of SRL and 20 ml H_2SO_4 was taken in kjedahl flask in which ½ digestion tablet (sodium sulphate + copper sulphate 10:1) was added in flask and was kept in digestion unit at 400^0C temperature for digestion. A colorless appearance shows complete digestion of protein. After complete digestion, the flask were left for cooling and transferred into the 50 ml volumetric flasks to make volume 50 ml. 5 ml of aliquot was distilled and distillate was collected in 2 % Boric acid. About 50 ml of distillate was titrated against 0.01 N H_2SO_4 to know the amount of total nitrogen present in sample.

TCA Precipitable Nitrogen and Non-protein Nitrogen:

For TCA precipitable nitrogen was determined according to the method of Targari et al., (1964). 5 ml of SRL was mixed with 5 ml 30 % TCA in test tube and kept for overnight. Then it was centrifuged at 4000 rpm for 30 minutes. 5 ml of supernatant was taken for nitrogen estimation by kjaldehl method. The nitrogen obtained in this fraction indicated non-protein nitrogen and subtraction from total nitrogen gave the TCA-precipitable nitrogen.

3.2.7 Enumeration of microbial population:

The rumen bacteria, protozoa and fungus were enumerated precisely as described earlier.

3.8 Digestibility /Metabolic trial:

After one month of experimental feeding with specific doses of antimicrobial agents, animals were placed in cages having facilities for quantitative collection of faeces and urine separately and a digestibility cum metabolic trial of 7 days collection period was conducted to study the role of different groups of microbes on nutrient utilization.

3.9 Chemical Analysis of feed, feces and urine:

Dry matter in feed and faeces was determined by oven drying at 100^0 C overnight. For chemical analysis sample of feed offered, refused and faeces were dried at 60^0C. Wet faeces and urine samples, preserved in concentrate H_2SO_4 and were analyzed for nitrogen. Feed and faeces samples were analyzed for dry matter, crude protein, ether extract, nitrogen free ether, crude fibre according to AOAC (1995). Fiber fractionation was done by following the method of Goering and VanSoest (1970).

Chapter 4

Result

4. RESULT

The microbiology of the different segments of the digestive tract, especially the rumen is extremely complex due to the large number of organisms present, the shifting populations that result from changes in the diet of host animal and the interaction among the various microbial groups / species. Although the development of the rumen microbial population is of interest from both physiological and ecological points of view, little is known about the microorganisms present in the pre-ruminants, the time establishment of the various microbial communities and the factors involved in the establishment of microorganisms necessary to carry out adequate ruminal fermentation. Moreover, the knowledge of establishment of anaerobic microbes will be of important to raise the young animals on fibrous diets in the way of cutting the cost of livestock production. The precise role of different microbial communities in local goat of Bundelkhand will also enrich the present knowledge status.

4.1 METABOLITES IN THE FOREGUT OF KID

Analysis of kid foregut content was carried out to know the various biochemical parameters and establishment of microbe.

4.1.1 pH:

The pH of stomach from birth to the fully functional rumen is presented in table 4.1. Immediately after birth, the stomach pH was found to be 5.04. On second week it was 6.23 and in the fourth week it reaches the ideal levels of 6.65. Thereafter, it reached up to 6.90 (maximum) in ninth week of age. Thus

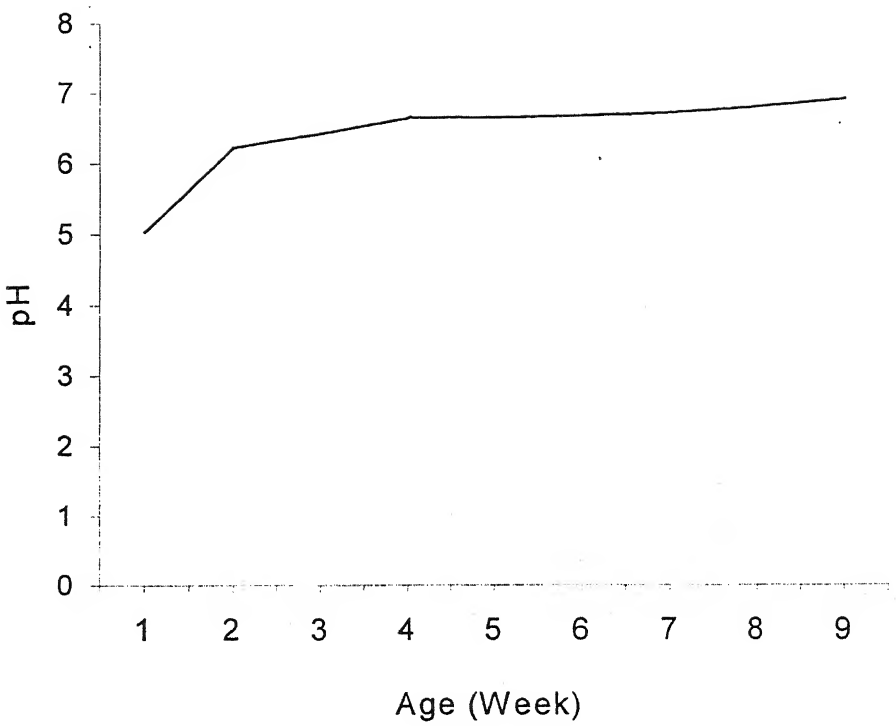


Fig. 4.1 pH of kid's foregut after birth

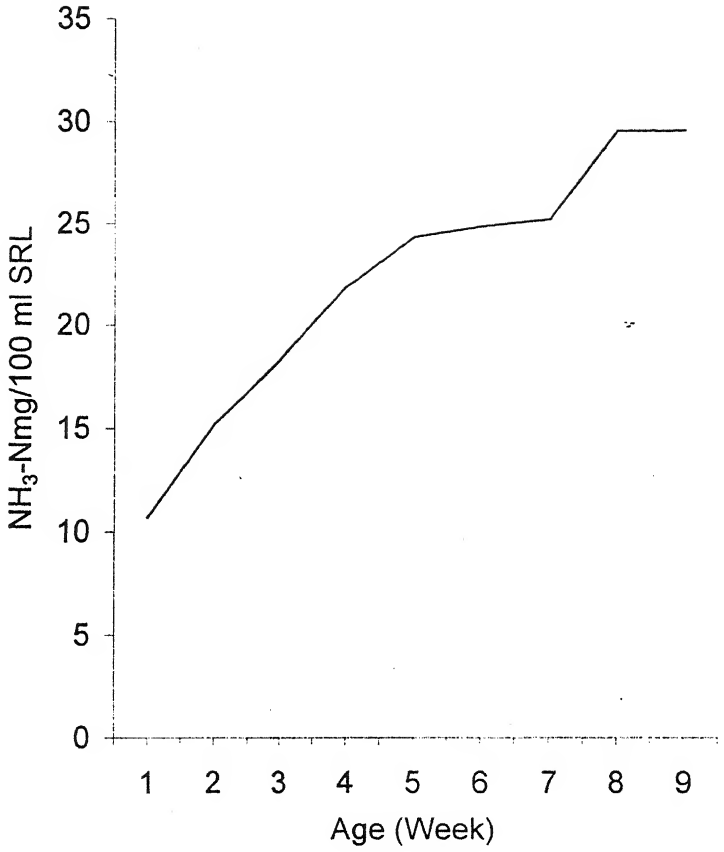


Fig. 4.2 NH3-N of kid's foregut after birth

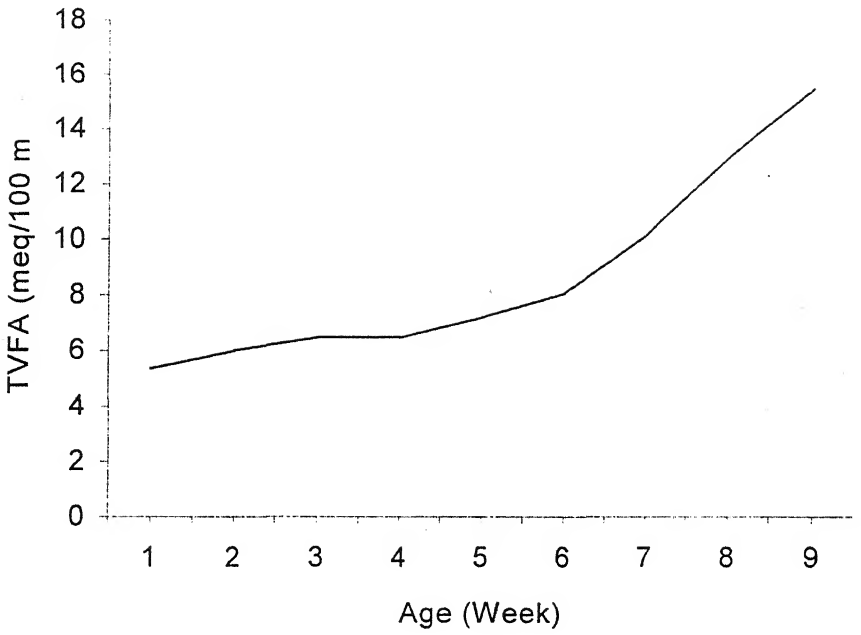


Fig. 4.3 TVFA of kid's foregut from birth onwards

the rumen pH is gradually moving towards alkaline side with the advancement of age (Figure 4.1).

4.1.2 Ammonia Nitrogen (NH_3-N):

Protein eaten by the animals converted into amino acid and then into ammonia nitrogen. This form of nitrogen is utilized by the microbes for their growth and multiplication. The ammonia nitrogen concentration in the foregut of kid is presented in Table 4.1. The concentration of ammonia nitrogen in first week was 10.71 mg/100 ml. In second week, it was 15.19 mg/100 ml, followed by 18.32 mg/100 ml in third week, 21.87 mg/100 ml in fourth week, 24.32 mg/100 ml in fifth week, 24.85 mg/100 ml in sixth week, 25.2 mg/100 ml in seventh week, 29.48 mg/100 ml in eighth week and 29.55 mg/100 ml in ninth week. This clearly indicated that with advancement of age activity of proteolytic enzyme increased as a result ammonia nitrogen concentration also increased (Figure 4.2).

4.1.3 Total volatile fatty acid (TVFA):

The concentrations of total VFA in foregut content of kids from birth to nine weeks of age is presented in Table 4.1. The total VFA concentration in first week was found to be only 5.38 meq/100ml. It was 6.03 meq/100ml, 6.49 meq/100ml 6.49meq/100ml, 7.21meq/100ml, 8.08 meq/100ml, and 10.14 meq/100ml from second to seventh week gradually. It was higher in eighth (12.94 meq/100ml SRL) and ninth week (15.42 meq/100ml SRL) of age. Upon perusal of Table 4.1, it was evidenced that anaerobic microbes of rumen clearly took the role of fermenting plant bio-molecules at the age of 7th week as during

Table 4.1 Metabolites in the foregut of kid from birth onwards

Age of kid (in Week)	Metabolites		
	pH	NH_3-N (mg/100 ml SRL)	TVFA (meq/100 ml)
1	5.04	10.71	5.38
2	6.23	15.19	6.03
3	6.43	18.32	6.49
4	6.65	21.87	6.49
5	6.65	24.32	7.21
6	6.67	24.85	8.08
7	6.71	25.2	10.14
8	6.79	29.48	12.94
9	6.90	29.55	15.42

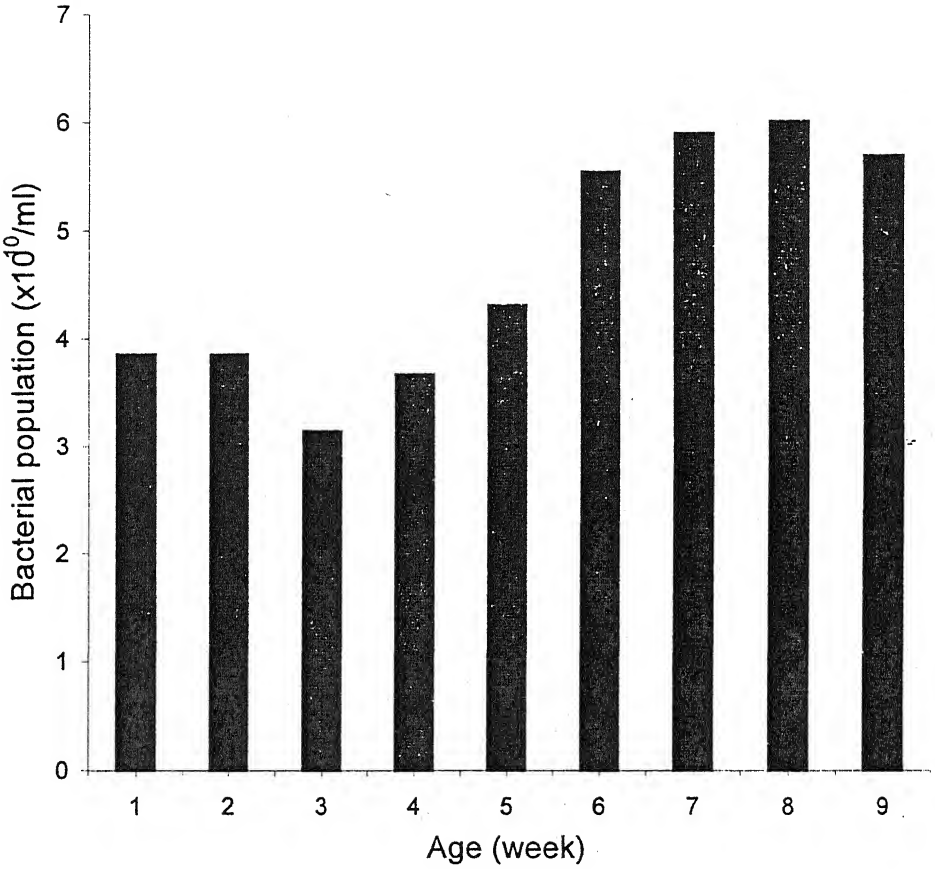


Fig. 4.4 Bacterial population of kid's foregut after birth

this age the total volatile fatty acid concentration reached up to 10.14 meq/100 ml. With the advancement of age rumen further developed as a result total volatile fatty acid concentration also increased (Figure 4.3).

4.2 ESTABLISHMENT OF MICROBES IN FOREGUT OF KID

4.2.1 Bacteria:

Bacterial population in kids was enumerated simply by counting colonies, which appeared in roll tubes after 1-2 days of incubation at 39^0C. The number of total viable bacteria in the foregut of kid from birth onwards is presented in Table 4.2. The bacteria population was lower during first three weeks of life and was 3.86×10^{10}/ml., 3.86×10^{10}/ml and 3.14×10^{10}/ml in first, second and third week, respectively. During fourth week the population was 3.67×10^{10} / ml. The population was 4.31×10^{10}/ ml in fifth week, 5.45×10^{10}/ml in sixth week, 5.89×10^{10} / ml in seventh week. It reached to peak 6.0×10^{10} / ml in eighth week and almost similar in ninth week of age (Figure 4.4).

4.2.2 Protozoa:

The establishment of protozoa in the foregut of kid is presented in Table 4.2 and 4.3. It was ample clear that protozoa started to appear in the foregut of kid from the second week of age. In the second week their total population was 7.04×10^4/ml SRL, which was comprised of Oligotrichs (7.04×10^4/ml) only with *Entodinia* (6.67×10^4/ml) (plate 1) and these *Entodinia* population was consisted of *E .bursa* (plate 2), *E. bimastus* (plate 3) and *E. rostralum* (plate 4) and *Polyplastron* (plate 5) (0.37×10^4 / ml). At the age of three weeks, there was a slight increase in total population (8.28×10^4/ ml) comprised of Oligotrichs

Table 4.2 Microbial population of kid foregut from birth onwards

Microbial population of foregut	Age (in weeks)								
	1	2	3	4	5	6	7	8	9
Total viable bacteria ($\times 10^{10}$/ml)	3.86	3.86	3.14	3.67	4.31	5.54	5.89	6.00	5.68
Total protozoa ($\times 10^{4}$/ml)	0	7.04	8.28	12.52	13.67	22.95	28.53	41.55	37.97
Total fungal population ($\times 10^{3}$/ml)	0.21	3.38	8.27	9.05	11.34	12.50	13.25	14.63	14.28

Table 4.3 Differential protozoal count of kid's foregut from birth onwards

Differential protozoa population ($\times 10^4$/ml)	**Age (in week)**								
	1	2	3	4	5	6	7	8	9
A. Oligotricha	nil	7.04	8.28	12.52	13.24	22.73	28.15	41.04	36.65
1.Entodinia	nil	6.67	6.76	8.65	9.77	18.50	21.37	32.07	34.13
2. Polyplastron	nil	0.37	1.11	1.61	1.79	1.01	1.89	2.32	0.55
3.Diplodinium	nil	nil	0.16	0.85	0.60	1.21	1.96	1.75	0.96
4. Eudiplodinium	nil	nil	0.25	1.23	0.79	1.69	1.33	2.24	0.88
5. Ophryoscolex	nil	nil	nil	0.13	0.29	0.32	1.60	1.66	0.13
B. Holotricha	nil	nil	nil	nil	0.43	0.22	0.38	0.51	1.32
1. Isotricha	nil	nil	nil	nil	nil	nil	nil	0.05	0.17
2. Dasytricha	nil	nil	nil	nil	0.43	0.22	0.38	0.46	1.15

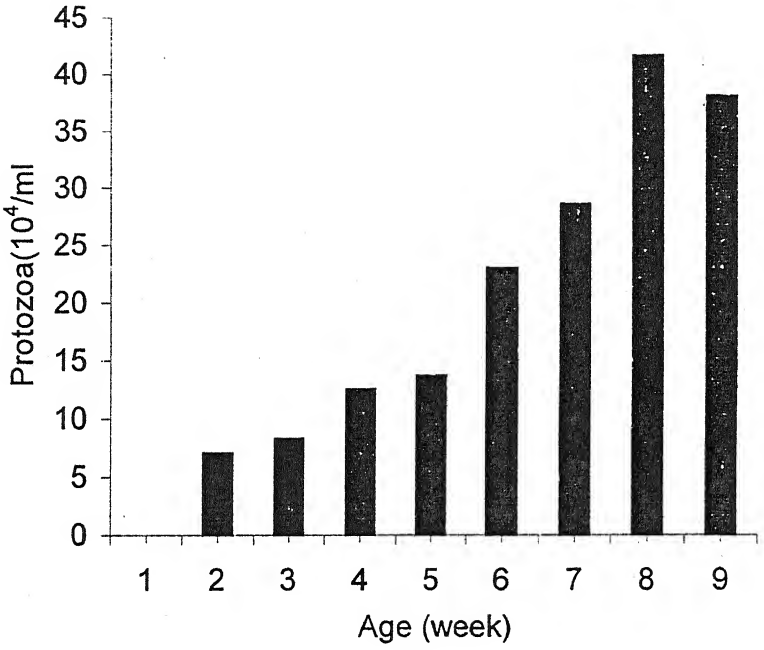


Fig 4.5 Total Protozoal population of kid's foregut from birth

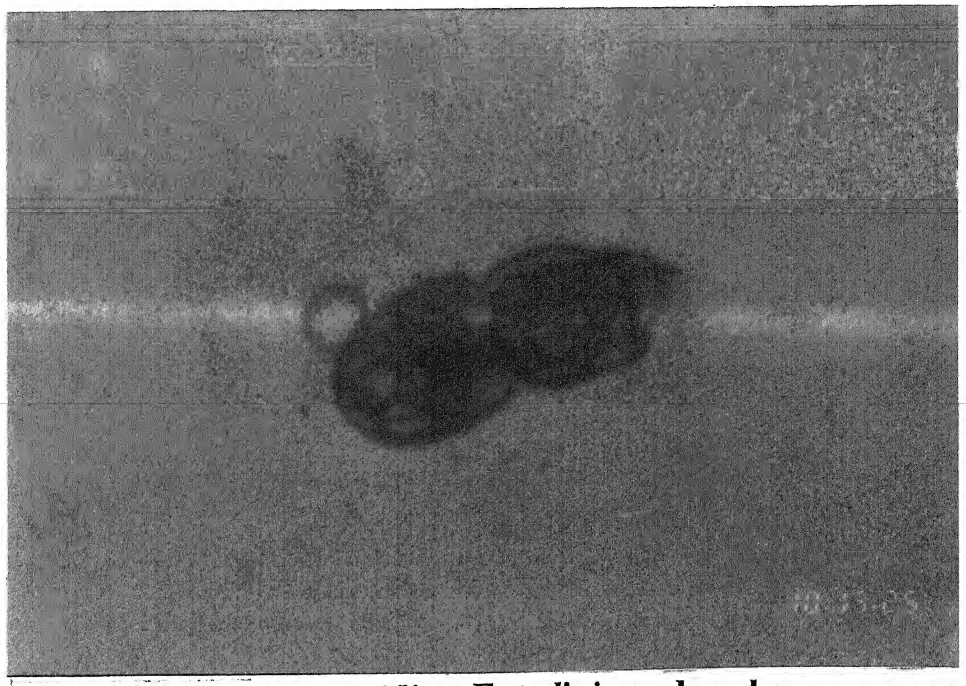


Plate No. 1 Dividing *Entodinia* under phase contrast microscope

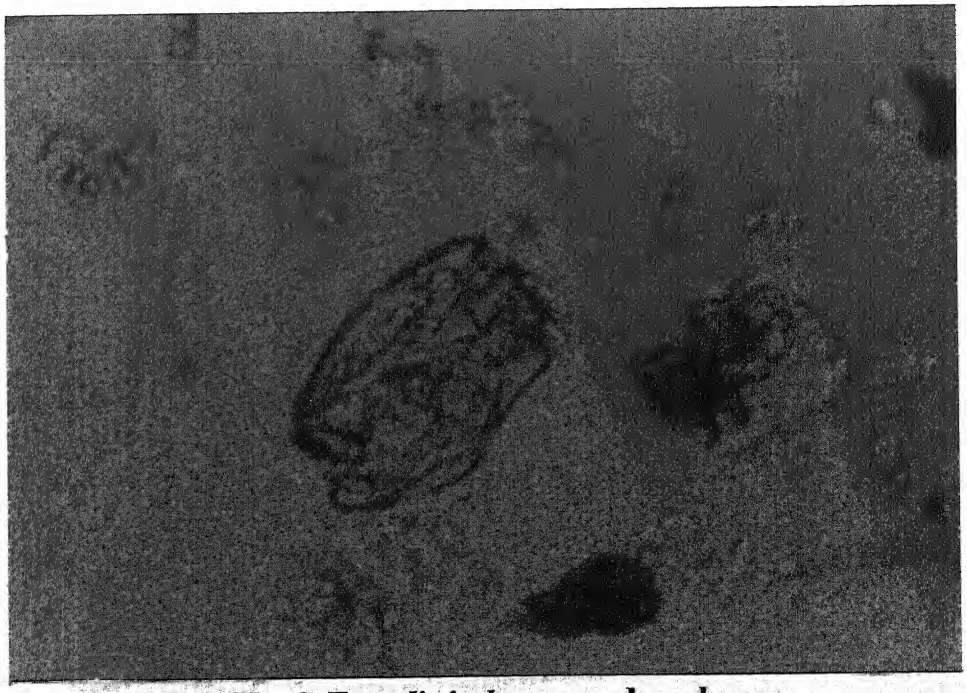


Plate No. 2 *Entodinia bursa* under phase contrast microscope

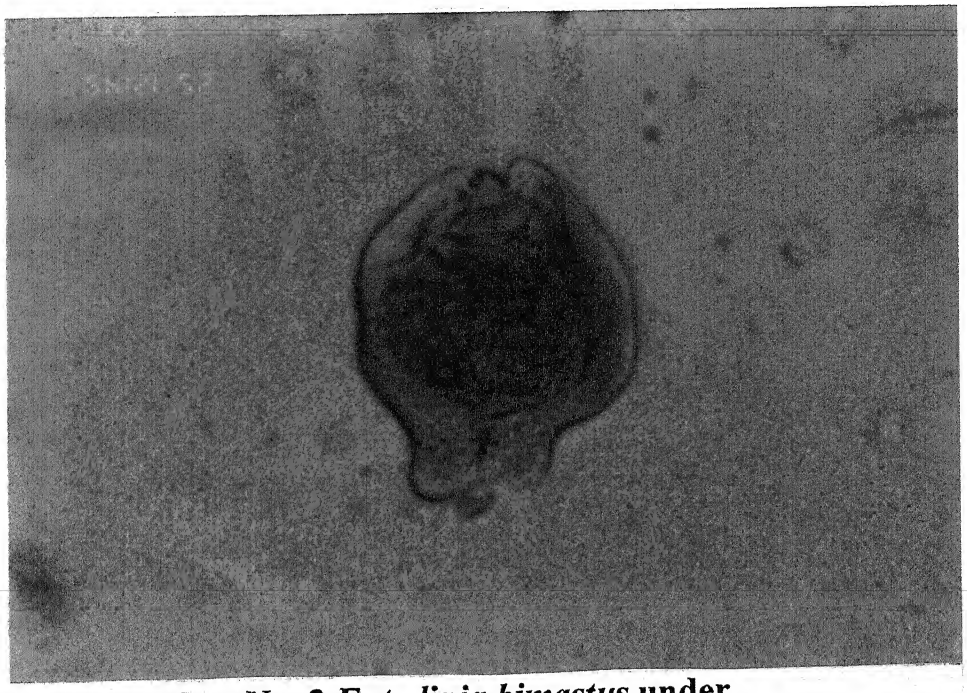


Plate No. 3 *Entodinia bimastus* under phase contrast microscope

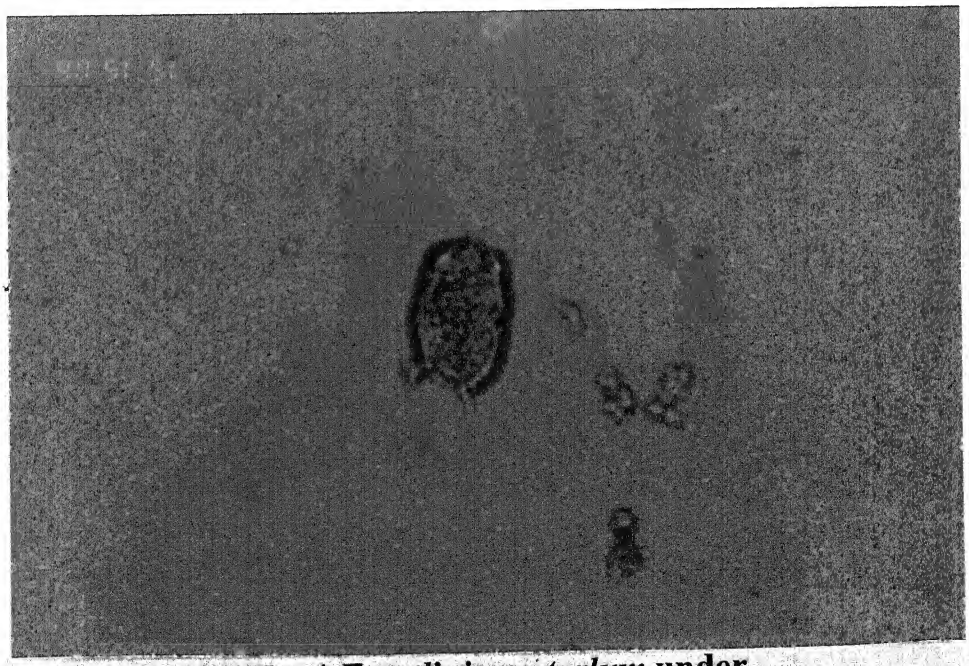


Plate No. 4 *Entodinia rostralum* under phase contrast microscope

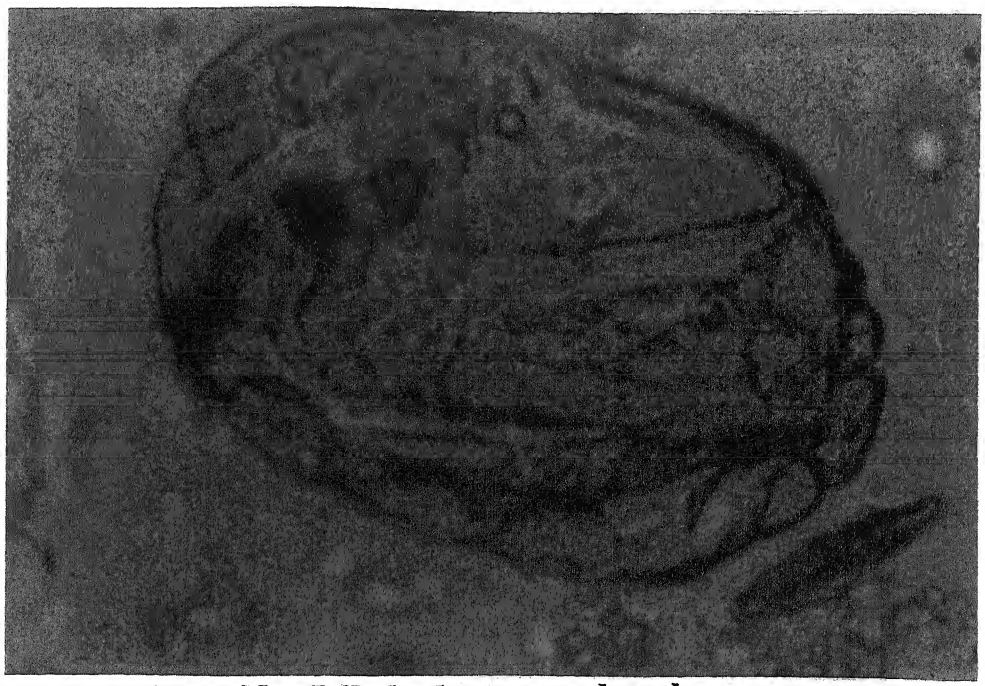


Plate No. 5 *Polyplastron* under phase contrast microscope

(8.28×10^4/ ml) with Entodinia (6.76×10^4/ ml), Polyplastron (1.11×10^4 / ml) and appearance of *Diplodinium* (0.16×10^4 / ml) was consists of *D. cristagalli* (plate 6) and *D. dentatum* (plate7) with *Eudiplodinium* (plate 8) (0.25×10^4/ ml). In fourth week total population was 12.52×10^4/ ml with one new genera of *Ophryoscolex* (plate 9) with 0.13×10^4/ml count. In the fifth week of age, total population was 13.67 $\times10^4$/ ml which was consisted of Oligotrichs (13.24×10^4/ ml) as well as Holotricha (0.43×10^4/ ml). The Holotricha included Dasytricha only (0.43×10^4 / ml). Similar genera were found in sixth and seventh week with total population 22.95×10^4/ ml and 28.53×10^4/ ml respectively which was consist of Oligotrichs as well as Holotricha. The protozoa population was higher in eighth week with total population of 41.55×10^4/ ml which was consisted of Oligotrichs (41.04×10^4/ ml) as well as Holotricha (0.51×10^4/ ml) with all previous genera and new genera *Isotricha* (0.05×10^4/). At end of experimental period, in ninth week, a slight decline (Figure 4.5) was found in total population (37.97×10^4 / ml) consisting of Oligotrichs (36.65×10^4/ ml) as well as Holotricha (1.32×10^4/ ml) with Isotricha (0.17×10^4/ ml) and Dasytricha (0.05×10^4/ ml).

Description of protozoa

A. Oligotricha

These were comparatively larger protozoa and cell surface did not possess uniform cilia. These were as follows:

1. **Entodinia**

This has only one oral ciliary band, no skeletal plates with average length 15-120 μm and average width 15-80 μm. Some species of *Entodinia* were

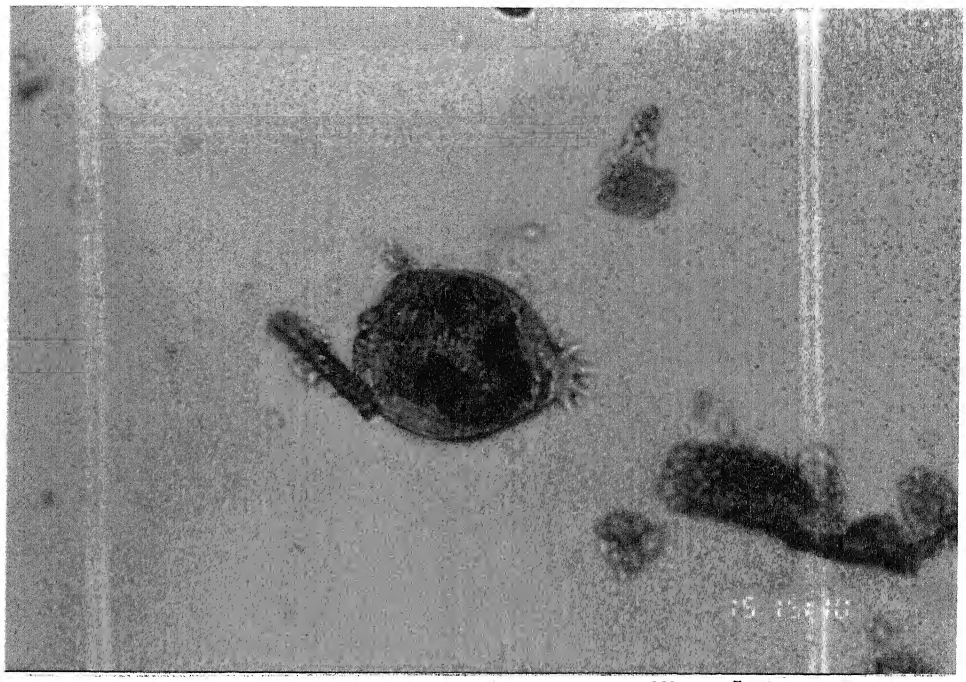


Plate No. 6 *Diplodinium cristagalli* under phase contrast microscope

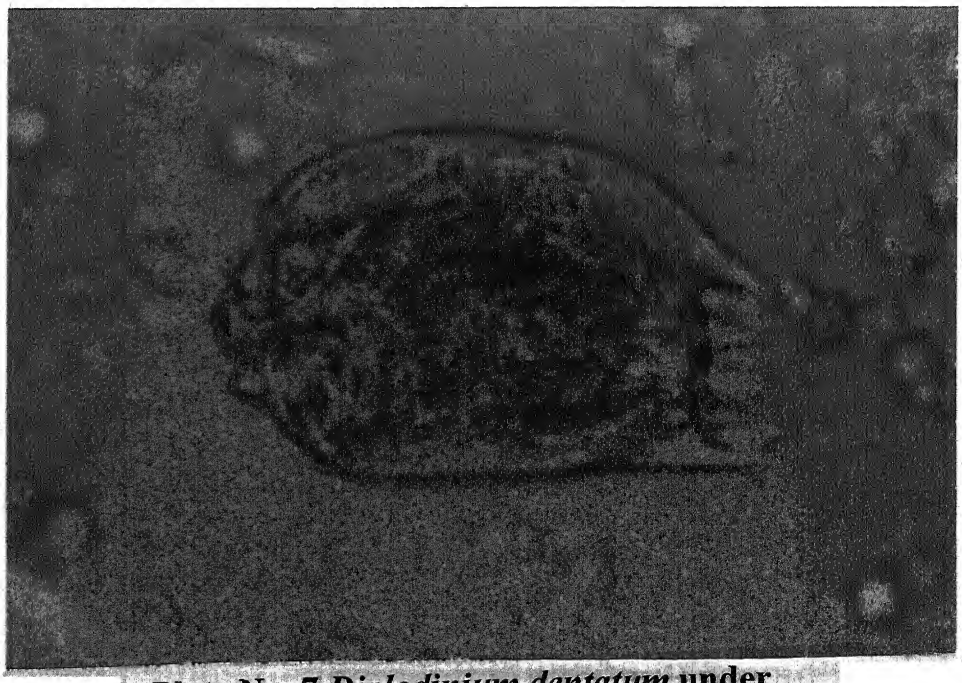


Plate No. 7 *Diplodinium dentatum* under phase contrast microscope

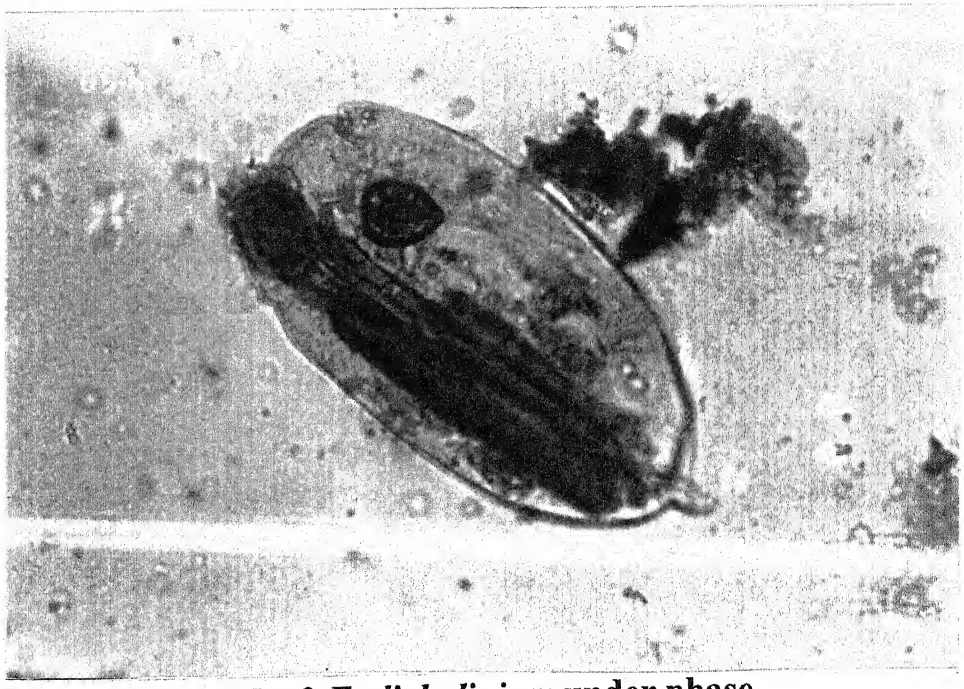


Plate No. 8 *Eudiplodinium* under phase contrast microscope

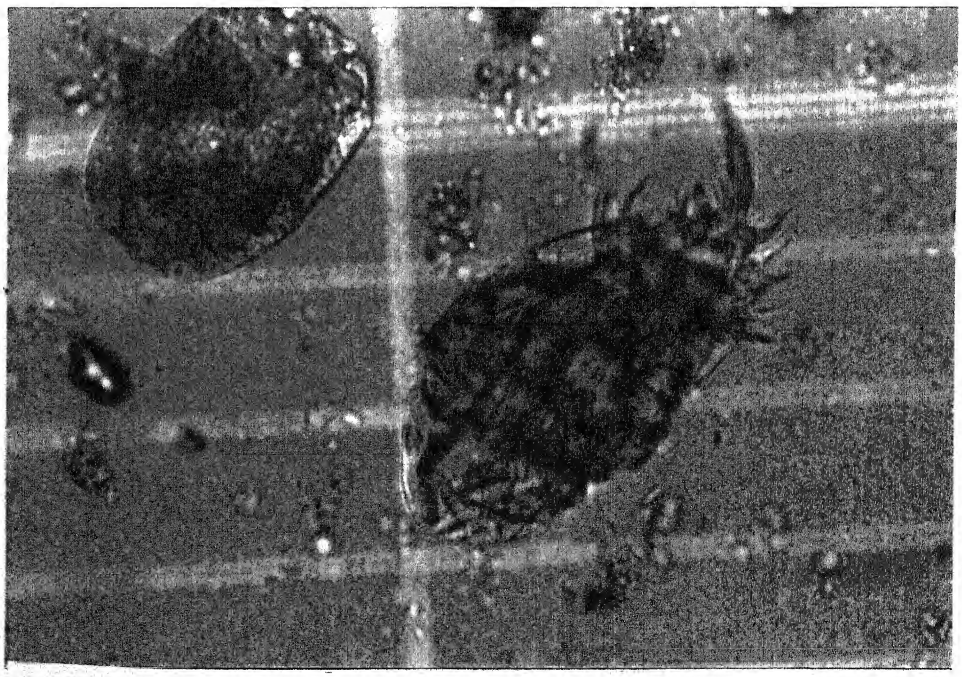


Plate No. 9 *Ophryoscolex* under phase contrast microscope

identified on the basis of above characters and size: *E. bimastus* , average length 35 – 54 μm and width 24 – 37μm, *E. bursa* with length 55-114 μm width 37 – 38μm and *E. rostralum* with average length 23-47 μm and width 23 –29 μm.

2. Diplodinium

Diplodinium was identified by the presence of two ciliary bands. One larger adoral band and another small oral band. No skeletal plates present. Its two species were identified as *D. crista-galli* with average length 55-120 μm, width 25-75 μm and *D. dentatum* with average length 65-82 μm and average width 40-50 μm.

3. Eudiplodinium

It had bands similar to *Diplodinium*. One dorsal and other adoral with one narrow skeletal plate. The size of the protozoa was 40-212 μm length and width 35-145μm.

4. Orphryoscolex

It had two ciliary zones; dorsal is displaced posteriorly to the middle of cell body. One long narrow caudal spine present in this protozoa. Skeletal plate fused. Its average length was 130-215μm and width 80-110μm.

5. Polyplastron

It had two ciliary bands, one dorsal and another adoral with five skeletal plates. Its average length was 161μm and width 95μm.

B. Holotrichs

These are comparatively smaller having cilia uniformly covering the body. These can swim more rapidly than Oligotrichs. These were of following two types:

1. Isotricha

Mouth on the posterior side of the body, with average length of 80-200 μm and width 45-150 μ m.

2. Dasytricha

Mouth on anterior side of the body, with average length of 45-100 μm and width 22-50μm.

4.2.3 Fungi:

Fungi are the new entrant in the domain of anaerobic microbiota. Earlier it was that fungi could not exist anaerobically. But the discovery of Orpin's flagellates in 1976 from the ovine rumen brought into notice that fungi can also exist anaerobically with the help of hydrogenosomes. Fungal population in kids was enumerated simply by counting colonies, which appeared in roll tubes after 2-3 days of incubation at 39^0C. The number of anaerobic fungi from birth onwards is presented in Table 4.2. Even in the first week of age, the fungal population was 0.21×10^3 / ml. At the age of second week it was 3.38×10^3 / ml, followed by 8.27×10^3/ ml in third week, 9.05×10^3/ ml in fourth week, 11.34×10^3/ ml in fifth week, 12.50×10^3/ ml in sixth week and 13.25×10^3/ ml in seventh weeks of age. It was highest in eighth week 14.63×10^3/ ml and almost similar in ninth week (14.28×10^3/ ml). Upon perusal of Table 4.2, it was clear

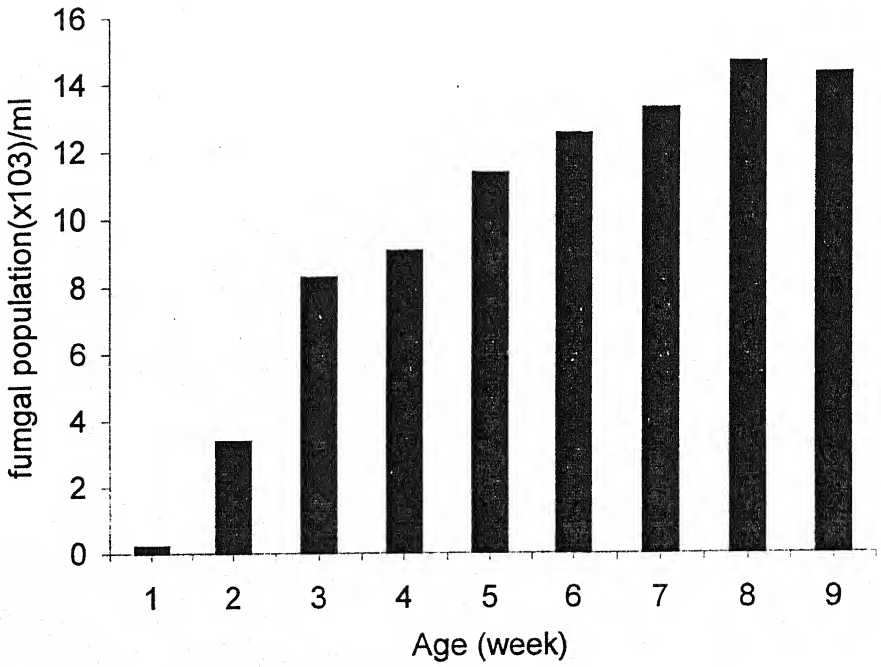


Fig. 4.6 Fungal population of kid's foregut from birth onwards

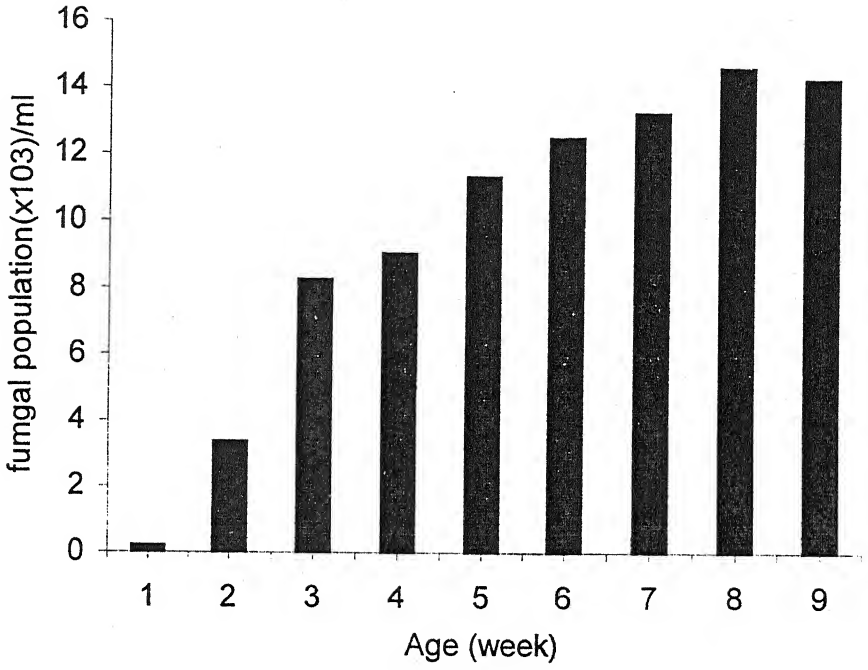


Fig. 4.6 Fungal population of kid's foregut from birth onwards

that population of rumen anaerobic fungus enhanced with the advancement of age (Figure 4.6).

To see the anaerobic fungus role on fiber digestion, rumen liquor was collected by stomach tube and observed directly the fiber particle under the phase contrast microscope. From the Plate 10, 11and 12, it was evidenced that anaerobic fungus actively participated in the mechanical breakdown of the fiber particles. The zoospores grown over the fiber particle and developed into matured sporangia with penetrating mechanical weapon of strong rhizomycelial network. It resulted breaking down of fiber particles into small pieces. It made accessible more surface areas for the action of chemical weapons i.e. enzymes secreted by bacteria, protozoa and fungi. Monocentric sporangia were found to attach with the fiber particles collected from the rumen.

4.3 DESCRIPTION OF FUNGAL ISOLATES OF KID'S FOREGUT

Fungal colonies developed in the roll tubes from the goat rumen were further grown in 0.3 % cellobiose soft agar to characterize morphologically (table 4.3) and to study the enzyme activities secreted by those fungal isolates. Based on rhizomycelial growth, zoospore morphology, zoospore flagellation, the fungal isolates were categorized into five viz.; GR_1, GR_2, GR_3, GR_4 and GR_5.

GR_1:

Single sporangium was found to develop from the thallus (plate 13). Therefore it was mono-centric in nature. Sporangia were small, circular and

Table 4.4 Characterization of anaerobic fungi isolated from goat rumen

Isolate	Nature of growth	Shape of sporangia	Rhizomycelia	Presence of stalk	Zoospore flagella	Name of species
GR_1	Monocentric	Circular	Unsegmented	Present	Monoflagellated	*Piromyces*
GR_2	Monocentric	Oval	Unsegmented	Present	Monoflagellated	*Piromyces*
GR_3	Monocentric	Oval	Unsegmented	Present	Monoflagellated	*Piromyces*
GR_4	Monocentric / Polycentric	Pear	Unsegmented	Absent	Mono flagellated	*Caecomyces*
GR_5	Monocentric	Dumbbell	Unsegmented	Present	Monoflagellated	*Piromyces*

possessed stalk at the base. Zoospores were mono-flagellated. Sporangium possessed un-segmented rhizomycelia. It appeared to be *Piromyces spp.*

GR_2:

Single sporangium developed from the thallus (plate14). Therefore it was mono-centric in nature. Sporangium was balloon shaped, circular at the top and tapered at the base (plate 14). Un-segmented extensively branched rhizomycelia was found with the sporangium. Zoospores were mono-flagellated. The isolate seemed to be *Piromyces spp.*

GR_3:

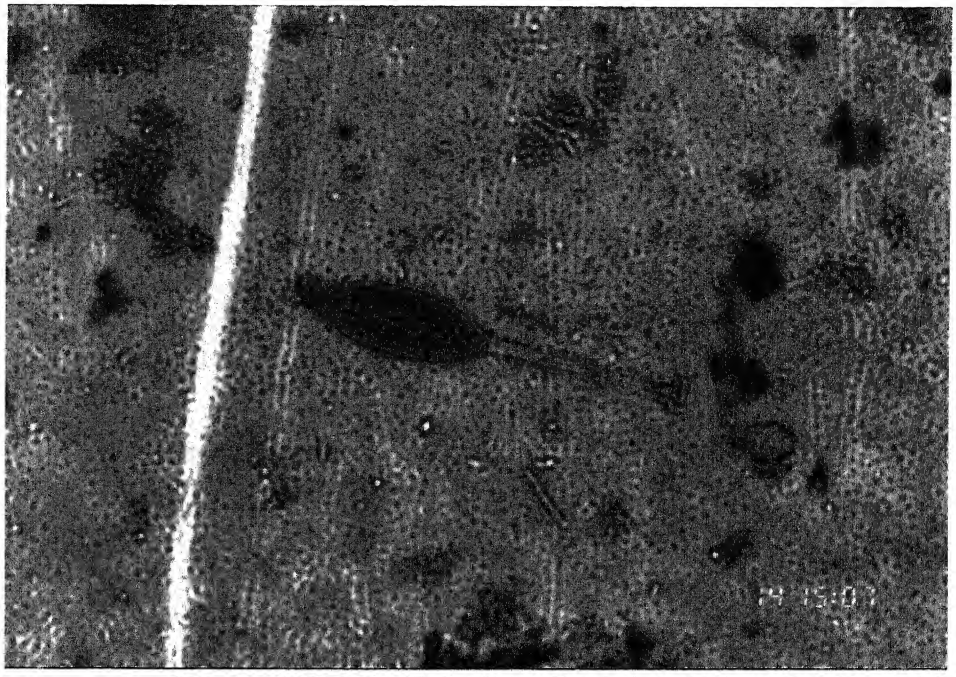
This isolate was mono-centric as single sporangium developed from the thallus. Sporangium was oval shaped (plate 15). Highly branched rhizomycelia developed from the sporangium. During zoosporogenesis, sporangial wall ruptured at one side and zoospore were released. Zoospores were mono-flagellated. It appeared to be *Piromyces spp.*

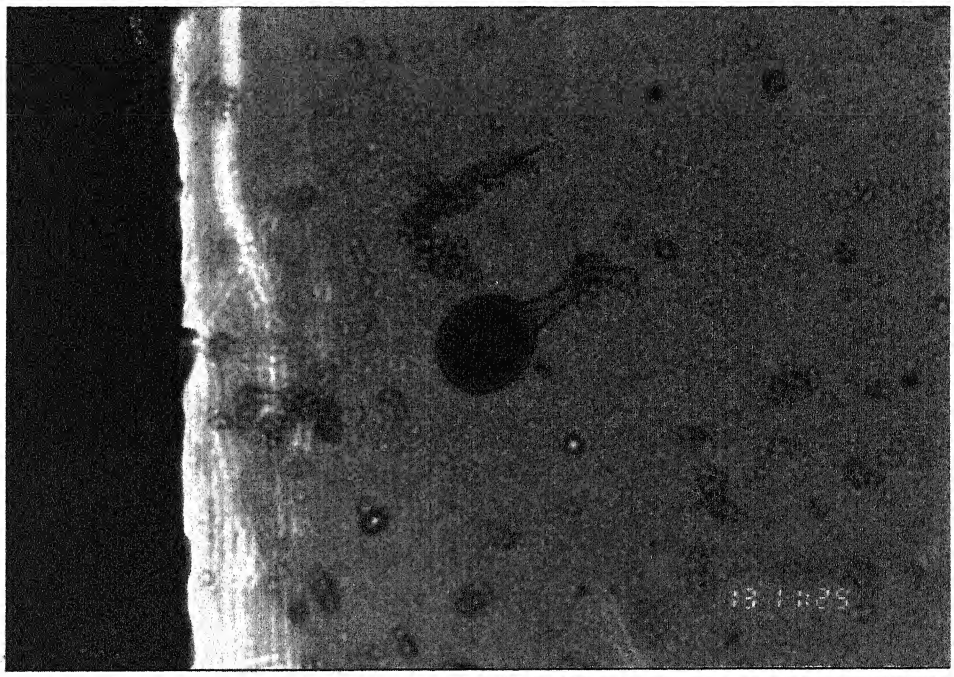
GR_4:

Sporangium was found single as well as in cluster. Pear shaped with bud like structure at one end of sporangium (plate 16). Both mono / polycentric in nature. Zoospores were mono-flagellated. The isolate seemed to be *Caecomyces spp.* This isolate did not possess any rhizomycelia.

GR_5:

Single sporangium developed from the thallus; therefore it was mono-centric in nature. At younger stages the sporangium were dumbbell shaped (plate 17). During maturity, sporangium elongated and looked like ovo-long.





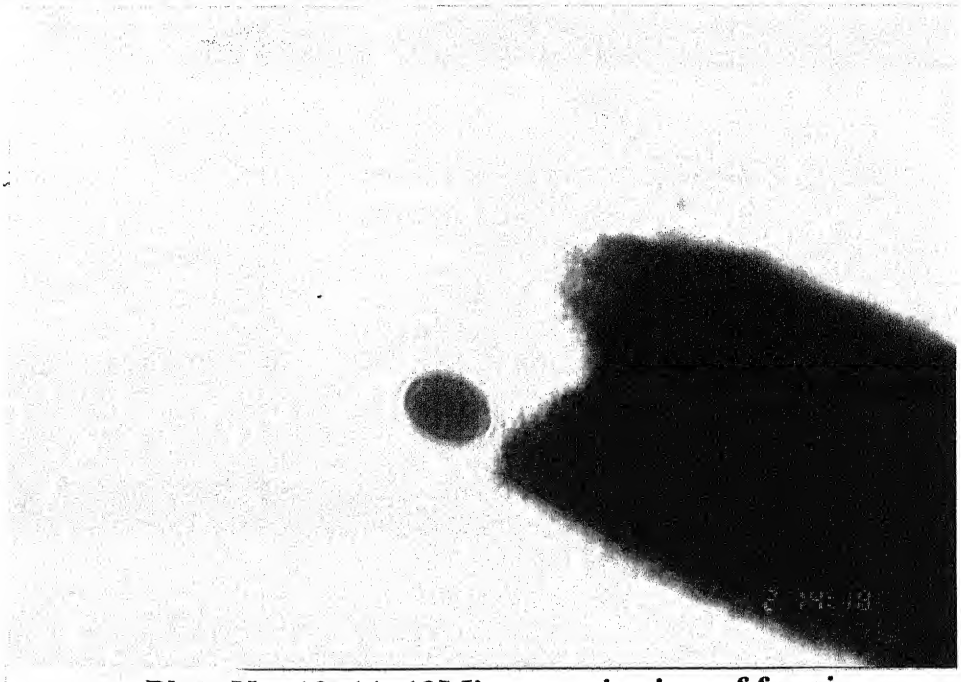


Plate No. 10, 11, 12Microscopic view of fungi direct attached with fungus

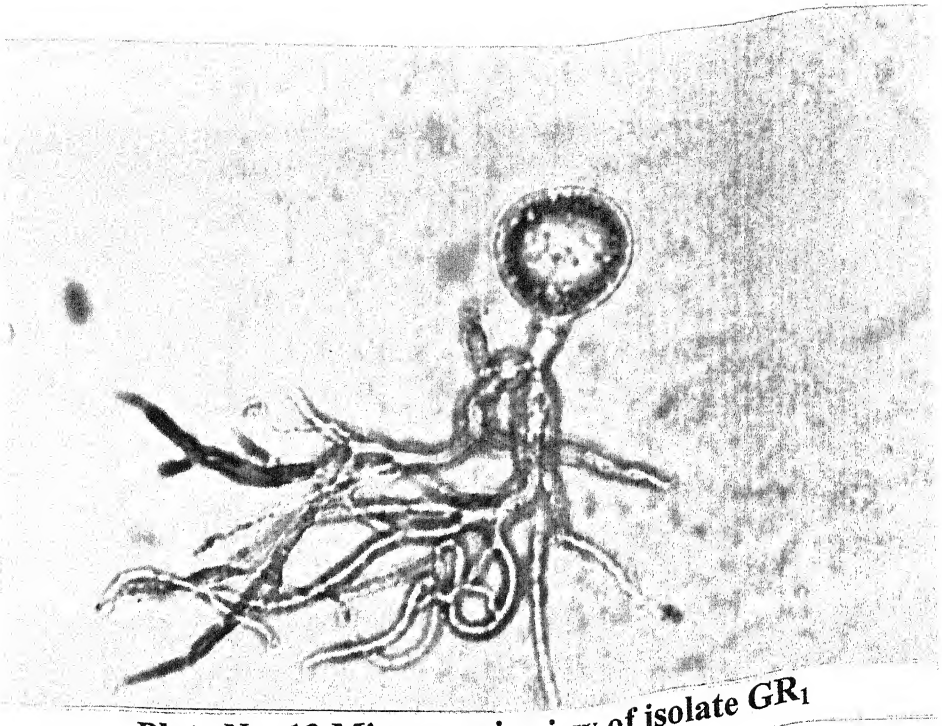


Plate No. 13 Microscopic view of isolate GR_1

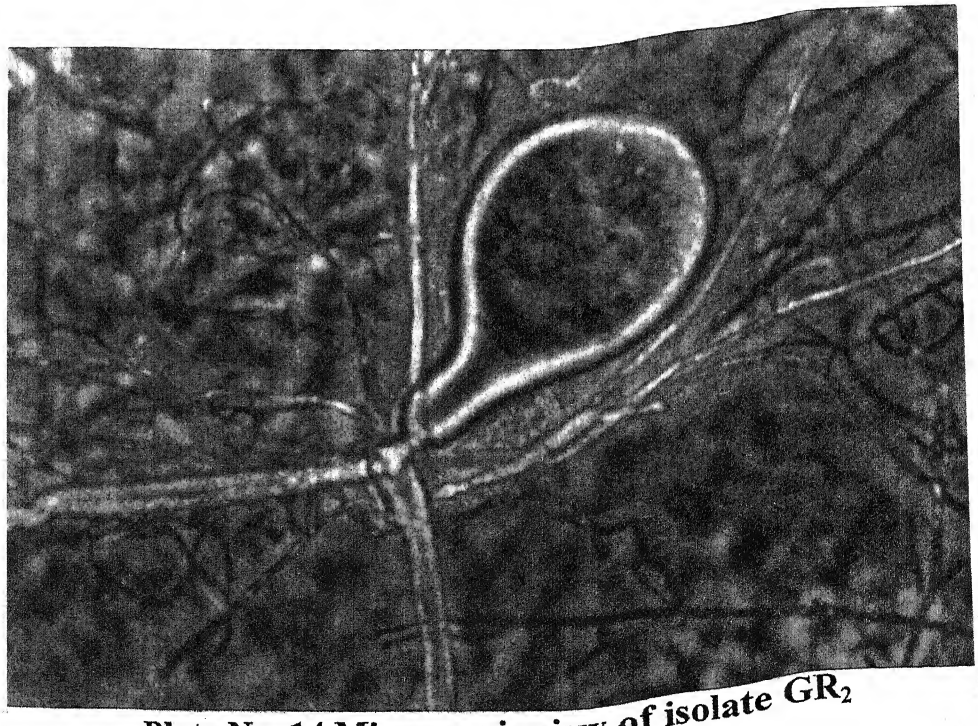


Plate No. 14 Microscopic view of isolate GR_2

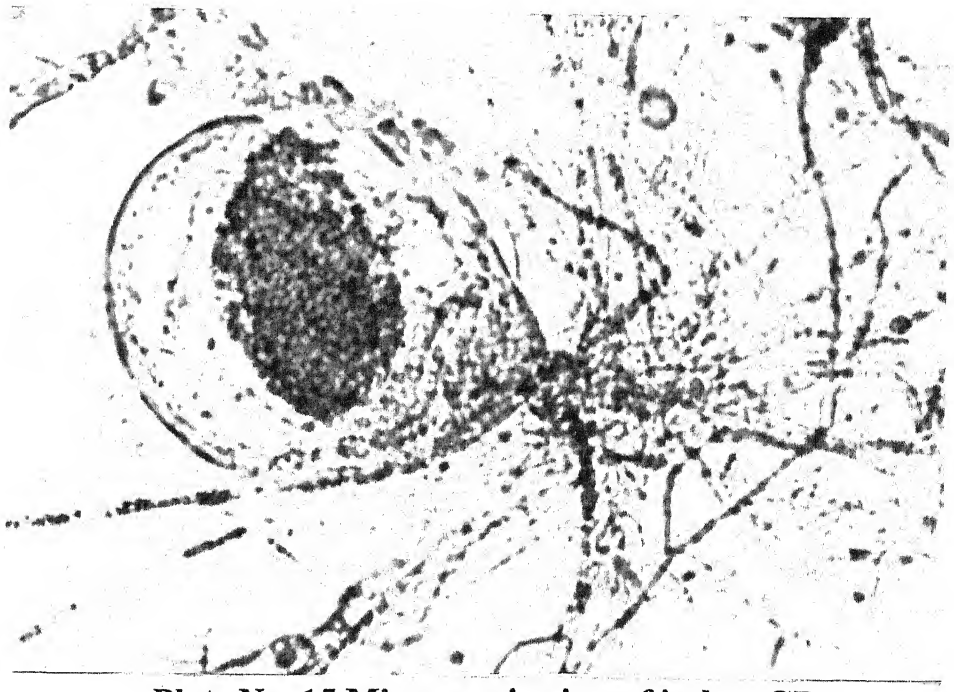


Plate No. 15 Microscopic view of isolate GR_3

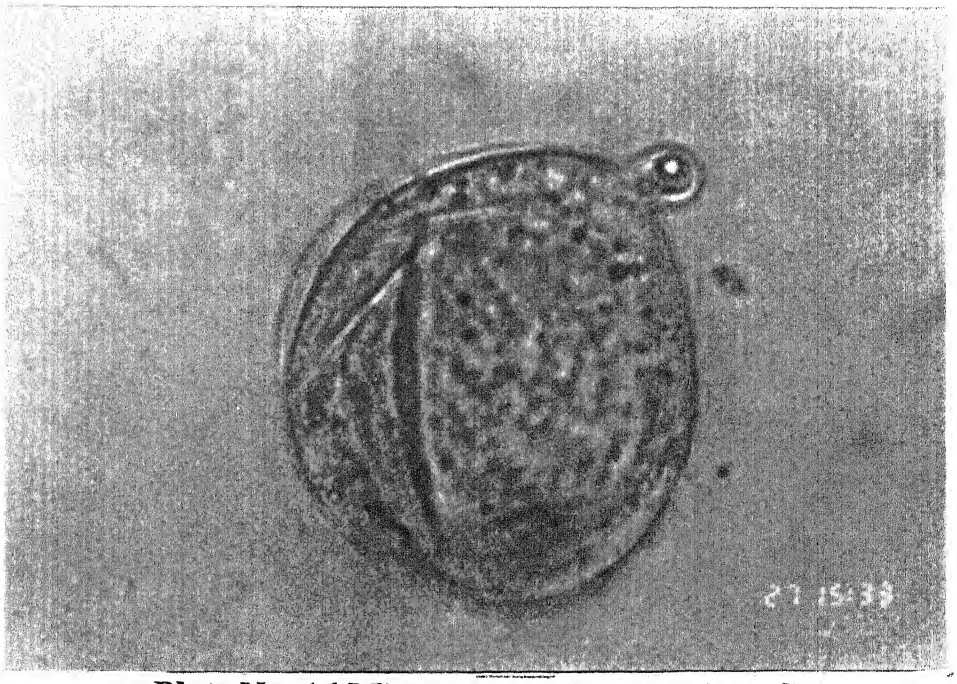


Plate No. 16 Microscopic view of isolate GR_4

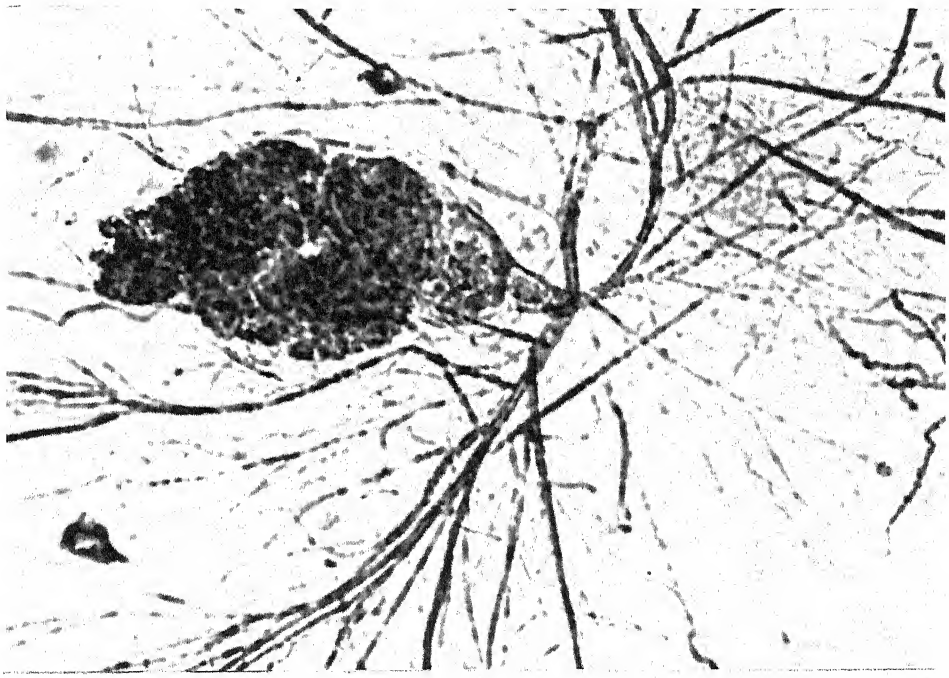


Plate No. 17 Microscopic view of isolate GR_5

Table 4.5 Metabolic product of goat fungal isolate

Fungal isolate	Acetate (µmole/ml)	Butyrate (µmole/ml)	Lactate (µg/ml)
GR_1	2.278	nil	38.88
GR_2	2.593	nil	21.84
GR_3	2.109	nil	32.64
GR_4	1.649	0.129	9.36
GR_5	2.770	0.137	22.08

Table 4.6 Plant cell wall degrading enzyme of anaerobic fungi isolate

Fungal isolate	Endoglucanase		FP-ase		Xylanase	
	Activity (mIU/ml)	Sp. act (IU/mg protein)	Activity (mIU/ml)	Sp. act (IU/mg protein)	Activity (IU/ml)	Sp. act (IU/mg protein)
Isolate GR_1	2.854	0.0049	-	-	0.0134	0.0233
Isolate GR_2	6.229	0.0071	3.208	0.0036	0.007	0.008
Isolate GR_3	5.400	0.006	3.004	0.0033	-	-
Isolate GR_4	5.204	0.0042	-	-	-	-
Isolate GR_5	5.825	0.0058	6.595	0.0065	0.024	0.024

Extensively branched rhizomycelia were seen in sporangium. The isolate seemed to be *Piromyces spp.* After maturity sporangial wall dissolved and the zoospore released from that area.

4.4 METABOLIC PRODUCTS OF ANAEROBIC FUNGAL ISOLATES

The metabolites produced by fungal isolates during growth in cellobiose soft agar media is presented in Table 4.5. The isolates viz.; GR_1, GR_2 and GR5 fermented cellobiose into both acetate and lactate. No butyrate was detected in their growth media. However, the isolates GR_4 and GR5 fermented cellobiose into acetate, butyrate and lactate. The acetate concentration in growth media ranged from 1.649 to 2.770 μ mole /ml. The butyrate concentration in the growth media of GR_4 and GR_5 ranged from 0.129 to 0.137 mole /ml. The highest level of lactate production was noticed in the case of anaerobic fungal isolate GR_1.

4.5 ENZYMES SECRETED BY FUNGAL ISOLATE

The plant cell degrading enzymes secreted by the anaerobic fungal isolates is presented in Table 4. 6. These included endoglucanase, FP-ase and xylanase. In GR_1, the concentration of endoglucanase and xylanase was 2.854 mIU/ml and 0.0134 IU/ml respectively. Their specific activity was 0.0049 IU/mg protein and 0.0233 IU/mg protein. No filter paper-ase and cellobiase activity was noticed in culture supernatant of the above isolates. In GR_2, endoglucanase was 6.229 mIU/ml, filter paper-ase was 3.208mIU/ml and xylanase was 0.007 IU/ml with their specific activities 0.0071 IU/mg protein and 0.0036 IU/mg protein and 0.008 IU/mg protein respectively. In GR_3 endoglucanase was 5.4 ml U/ml, filter paper-ase was 3.004mIU/ml and no xylanase and cellobiase were detected. The

specific activities were 0.006 IU/mg protein, 0.0033 IU/mg protein for endoglucanase and FP-ase respectively. GR_4 isolate produced only endoglucanase, its concentration was 5.204mIU/ml with specific activity 0.0042 IU/mg protein. No filter paper-ase, xylanase and cellobiase were detected in culture supernatant. In GR_5, the endoglucanase was 5.825mIU/ml, FP-ase was 6.595mIU/ml and xylanase was 0.024 IU/ml with specific activity of 0.0058 IU/mg protein, 0.0065 IU/mg protein and 0.024 IU/mg protein respectively.

4.6 EFFECT OF ANTIMICROBIAL AGENTS ON RUMEN METABOLITES OF GOAT

To define the role of different groups of microbes, antimicrobial agents against specific groups of microorganism was given orally to the adult goats. The group T_1 was used as control where rumen fermentation was continued by the presence of bacteria, protozoa and fungi. In the case of T_2, protozoa were removed by oral dosing of sodium lauryl sulphate. This treatment represented the rumen fermentation by bacteria and fungi only. In the case of T_3, anaerobic fungi were removed by oral dosing of fluconazole capsule. The rumen fermentation of these goats was carried out by bacteria and protozoa only. In the T_4 treatment, bacteria population was suppressed by oral dosing of broad-spectrum (Tetracycline) antibiotics. These groups of goats represented the rumen fermentation by the active role of fungi and protozoa. The effect of various antimicrobial agents on rumen metabolites is presented in Table 4.7.

4.6.1 pH:

The pH of the SRL was recorded immediately after collection of rumen liquor from adult goats using a digital pH meter, which was first standardized by standard buffer solution. The rumen pH in control goats was 6.91 while it was 6.87 in T_2, 6.64 in T_3 and 6.95 in T_4. The pH did not affected by removal of microbial communities and remained identical in all the groups.

4.6.2 Total volatile fatty acid:

The structural and storage carbohydrates are metabolized in the rumen in to different volatile fatty acids viz.; acetic acid, propionic acid and butyric acid. The concentrations of total volatile fatty acids were 14.32, 13.82, 14.53 and 7.53 (meq/100 ml) respectively from T_1, T_2, T_3 and T_4 respectively. It was almost identical in three groups (T_1, T_2 and T_3) while it was lowest in T_4 where bacteria population was suppressed by Tetracycline. The removal of protozoa and fungus did not affect the total volatile fatty acid concentration as the role was taken care of by enhanced bacterial population.

4.6.3 Ammonia nitrogen:

As a result of strong proteolytic activity of the rumen microbes, the protein and non-protein nitrogenous compounds are degraded into amino acids and subsequently into ammonia. This ammonia nitrogen is being used for microbial protein synthesis which further passes into the lower tract for digestion and absorption in the form of amino acids. The concentration of ammonia nitrogen was analyzed in all treatment groups. It was 19.71 mg/100ml in T_1, 15.15 mg/100ml in T_2, 18.53 mg/100ml in T_3 and 10.53 mg/100ml T_4

groups. Therefore, ammonia nitrogen concentration was highest in control goats and lowest in the goats where fermentation was carried out only by protozoa and fungi i.e. T_4.

4.6.4 Total nitrogen:

The total nitrogen includes all forms of nitrogen in the rumen ecosystem. This is classified into two viz.; true protein or TCA precipitable nitrogen and non-protein nitrogen. The concentration of total nitrogen in treatment groups were 64.63 mg/100 ml, 60.3 mg/100 ml, 60.57 mg/100 ml and 40.57 mg/100 ml in T_1, T_2, T_3 and T4 respectively. It was lowest in T_4 goats where rumen bacteria were suppressed by antibiotics and fermentation was carried out only by protozoa and fungi. Total nitrogen was found to be highest were rumen fermentation in goats were undergone by the action of all the microbial communities i.e. bacteria, protozoa and fungi.

4.6.5 Non protein nitrogen:

The concentration of non protein nitrogen is presented in Table 4.7. The highest concentration of non-protein nitrogen was noticed in T_1 followed by T_2, T_3 and T_4. The NPN concentrations in respective groups of goats were 35.11 mg/100 ml in T_1, 31.61 mg/100 ml in T_2, 29.84 mg/100 ml in T_3 and 13.36 mg/100 ml in T_4.

4.6.6 TCA ppt nitrogen:

The concentration of TCA ppt nitrogen in goats is presented in Table 4.7. The true protein or TCA precipitable nitrogen was highest in T_3 where fermentation was carried out by the only bacteria and protozoa and fungus was

Table 4.7 Effect of antimicrobial agents on rumen metabolites

Group	Control (T_1)	Defaunated (T_2)	Fungus free (T_3)	Bacteria free (T_4)
pH	6.91	6.87	6.64	6.95
VFA (meq/100 ml)	14.32	13.82	14.53	7.53
NH_3-N (mg/100ml)	19.71	15.15	18.53	10.53
Total nitrogen (mg/100ml SRL)	64.63	60.3	60.57	40.57
Non protein nitrogen (mg/100ml SRL)	35.11	31.61	29.84	13.36
TCA ppt nitrogen (mg/100ml SRL)	28.52	28.69	30.73	27.21

T_1 : Presence of bacteria, protozoa and fungi

T_2 : Removal of protozoa by sodium lauryl sulphate

T_3 : Removal of fungus by flucanazole

T_4 : Removal of bacteria by tetracycline

Table 4.8 Effect of antimicrobial agents on rumen microbial population of goat

GROUP	Control (T_1)	Defaunated (T_2)	Fungus free (T_3)	Bacteria free (T_4)
Bacteria ($x10^{10}$/ml)	21.87	36.83	32.53	REMOVED
Fungi ($x10^2$/ml)	24.83	36.25	REMOVED	34.0
Total Protozoa (x 10^5/ml)	4.46		8.2	5.41
Oligotricha (x 10^5/ml)	4.12	R	6.45	5.07
Entodinia (x 10^5/ml)	3.48	E	4.2	4.63
Polyplastron (x 10^5/ml)	0.17	M	0.32	0.19
Eudiplodinium (x 10^5/ml)	0.11	O	0.49	0.09
Ophryoscolex (x 10^5/ml)	0.05	V	0.31	0.08
Diplodinium (x 10^5/ml)	0.31	E	1.13	0.08
Holotricha (x 10^5/ml)	0.34	D	1.75	0.34
Isotricha (x 10^5/ml)	0.25		1.29	0.2
Dasytricha(x 10^5/ml)	0.09		0.46	0.14

T_1 : Presence of bacteria, protozoa and fungi

T_2 : Removal of protozoa by sodium lauryl sulphate

T_3 : Removal of fungus by flucanazole

T_4 : Removal of bacteria by tetracycline

eliminated by Flucanozole capsules. This was followed by T_2 and T_1 where almost identical levels of TCA precipitable nitrogen concentration were noticed. The least concentration of TCA precipitable nitrogen was recorded in T_4 because of absence of important member of rumen ecosystem i.e. bacteria. In T_4, bacteria population was suppressed by Tetracycline and rumen fermentation was continued with the active role of protozoa and fungi. The TCA precipitable nitrogen concentrations were 28.52 mg/100 ml in T_1, 28.69 mg/100 ml in T_2, 30.73 mg/100 ml in T_3 and 27.21 mg/100 ml in T_4 groups.

4.7 EFFECT OF ANTIMICROBIAL AGENTS ON RUMEN MICROBIAL POPULATION IN GOAT

The effect of various antimicrobial agents on microbial population has been presented in Table 4.8

4.7.1 Bacteria:

Bacterial population was enumerated simply by counting colonies, which appeared in roll tubes after 1-2 days of incubation at 39^0C. The total viable bacteria population was 21.87×10^{10} / ml in T_1, 36.83×10^{10} / ml in T_2, 32.53×10^{10} / ml in T_3 and T_4 was free from bacteria. This indicated that removal of protozoa (defaunation) from the rumen significantly enhanced the bacteria population. Removal of fungus also attributed to the higher total viable bacteria in the rumen of goat.

4.7.2 Protozoa:

In T_1 group total protozoa population was 4.46×10^5/ml, which consisted of Oligotricha (4.12×10^5/ml) represented by *Entodinia* 3.48×10^5 / ml, *Polyplastron*

0.17×10^5 / ml, *Eudiplodinium* 0.11×10^5/ ml, *Ophryoscolex* 0.05×10^5/ml and *Diplodinium* 0.31×10^5/ml and Holotricha (0.34×10^5/ml) represented by *Isotricha* 0.25×10^5 / ml and *Dasytricha* 0.09×10^5 / ml. In T_2 defaunated, there were no ciliate observed in this group. This was protozoa free. In T_3 total protozoa population was 8.2×10^5/ ml; consisting of Oligotricha (6.45×10^5 / ml) with *Entodinia* 4.2×10^5 / ml, *Polyplastron* 0.32×10^5/ ml, *Eudiplodinium* 0.49×10^5/ml, *Ophryoscolex* 0.31×10^5 / ml, *Diplodinium* 1.13×10^5 / ml, and Holotricha (1.75×10^5 / ml) with *Isotricha* 1.29×10^5 / ml and *Dasytricha* 0.46×10^5 / ml and in T_4 group total population 5.41×10^5/ml which possessing both Oligotricha (5.07×10^5/ml) with *Entodinia* 4.63×10^5/ml, *Polyplastron* 0.19×10^5/ml, *Eudiplodinium* 0.09×10^5/ ml, *Ophryoscolex* 0.08×10^5/ ml, *Diplodinium* 0.08×10^5 / ml and Holotricha (0.34×10^5 / ml) with *Isotricha* 0.2×10^5 / ml and *Dasytricha* 0.14×10^5/ ml. Upon perusal of Table 4.8, it was evidenced that removal of fungus or suppression of bacteria enhanced the total protozoa population than control groups. The increase in protozoa number was attributed both by Oligotricha as well as Holotricha.

4.7.3 Fungi:

Enumeration of anaerobic fungal population was done by counting the colonies appeared in the roll tubes after 2-3 days of incubation at 39^0C. The number of anaerobic fungi was 24.83×10^2 / ml in T_1. Defaunation lead to increase in the number of anaerobic fungus and it reached up to 36.25×10^2 / ml (T_2). Similarly suppression of bacteria (T_4) also enhanced the fungal population and it reached up to population of 34.0×10^2 / ml.

Table 4.9 **Effect of antimicrobial agents on DMI and nutrient digestibility in goat**

Particulars	T1	T2	T3	T4
Body weight (kg)	26.0 ±1.7	24.6 ±1.62	22.0 ±1.68	22.6 ±1.8
DM intake (g)	582.6	700.7	561.1	406.6
DMI % b. wt.	2.24	2.85	2.5	3.0
DMI (g/w $^{0.75}$)	50.61	63.46	55.22	39.25
CP intake (g/day)	86.69	86.09	84.53	67.29
Nutrient digestibility				
DM	68.14	66.95	63.63	58.92
OM	70.43	65.66	64.10	60.34
NDF	55.86	55.31	52.50	50.91
ADF	44.08	43.58	37.93	36.54
CP	70.28	71.48	69.23	63.54
CF	65.83	63.66	60.09	56.40
EE	66.46	65.32	66.12	61.73
NFE	68.81	66.41	64.72	62.29

T_1 : Presence of bacteria, protozoa and fungi

T_2 : Removal of protozoa by sodium lauryl sulphate

T_3 : Removal of fungus by flucanazole

T_4 : Removal of bacteria by tetracyclin

Table 4.10 Total effect of antimicrobial agents

Effect	Defaunation	Fungus free	Bacteria free
Decreased	TVFA, NH3-N, TN, OM, CF, NFE	PH, TN, NPN, DM intake, CP intake, DM, OM, NDF, ADF, CF, NFE	TVFA, NH3-N, TN, NPN, DM intake, DMI, CP intake, OM, NDF, ADF, CP, EE, NFE
Increased	Bacterial and fungal population	TCA ppt. N, bacterial and protozoa population, DMI% b.wt,	Protozoa and fungal population
No significant effect	pH, TCA ppt. N, CP intake, NDF, ADF,EE	NH3-N, TVFA, CP, EE	pH, TCA ppt. N

4.8 EFFEECT OF ANTIMOCROBIAL AGENTS ON DMI AND NUTRIENT UTILIZATION IN GOAT

The goats were housed in groups following application of different anti microbial agents to study the role of different groups of microbes on nutrient utilization. The average body weight of goats in different groups were 26.0±1.7 kg in T_1, 24.6±1.62 kg in T_2, 22.0 ±1.68 kg in T_3 and 22.6 ±1.8 kg in T_4. The dry matter intake in different groups was 582.6, 700.7, 561.1 and 406.6 g /day in T_1, T_2, T_3 and T_4 respectively. The dry matter intake in terms of percentage body weight were 2.24 in T_1, 2.85 in T_2, 2.50 in T_3 and 3.0 in T_4. When dry matter intake was expressed in terms of gram per kg metabolic body size, the values were 50.61g / $W^{0.75}$ in T_1, 63.46 g / $W^{0.75}$ in T_2, 55.22 g / $W^{0.75}$ in T_3 and 39.25 61g / $W^{0.75}$ in T_4. The crude protein intake (g /day) in goats were 86.69 g in T_1, 86.09 g in T_2, 84.53 g in T_3 and 67.29 g in T_4. The Dry matter digestibility ranged from 58.92 to 76.95 % being highest in T_2 followed by T_1, T_3 and T_4. The organic matter digestibility was found to be highest (70.43 %) in T_1 and it was lowest in T_4 where bacteria population was suppressed by antibiotics. The neutral detergent fiber digestibility ranged from 50.91 to 55.86 %. The higher neutral detergent fiber digestibility was noticed in goats where rumen fermentation was carried out by combined activity of bacteria, protozoa and fungi. The suppression of bacteria population significantly inhibited the digestibility of neutral detergent fiber. The acid detergent fiber digestibility varied from 36.54 to 44.08 % and it was highest in T_1 followed by T_2, T_3 and T_4. The crude protein digestibility was not much affected by either removal of

protozoa or fungi but it lowered significantly in the goats where bacteria population was suppressed. The crude fiber digestibility ranged from 56.40 to 65.83 %, being highest in T_1 followed by T_2, T_3 and T_4. The ether extract digestibility did not differ much among the various treatment groups and varied from 61.73 to 66.46 %. The digestibility coefficient of nitrogen free extract was noticed to vary from 62.29 to 68.81 %, being highest in T_1 followed by T_2, T_3 and T_4.

Chapter 5

Discussion

5. DISCUSSION

Goats depend for their survival on the symbiotic association with the diverse groups of microorganisms present in the alimentary tract. The diet of this small ruminant is comprised of plant structural carbohydrates such as cellulose and hemicellulose. The animals themselves are unable to digest those plant biomolecules by their own enzyme systems. Therefore, they solely depend upon the anaerobic microorganisms (bacteria, protozoa and fungi) of the alimentary tract for the hydrolysis of plant biomolecules for entrapping the energy, protein, vitamins and minerals. As result of anaerobic fermentation, volatile fatty acids, vitamins, microbial proteins, different gases are produced in the rumen for their further utilization at the lower tract. In organized farm, weaning is done to conserve the milk for human consumption and introduction of fibrous feed at early ages. The alimentary canal of newborn animal is sterile and similar to monogastric. Therefore, it is utmost important to know the exact time of establishment of different microbes so that early incorporation of fibrous diets does not affect the growth of animals. Hence the present experiment was conducted to know the date of establishment of bacteria, protozoa and fungi in local goats. Moreover, the local goats are less studied for defining the role of different groups of microbes on nutrient utilization. Therefore, the studies also encompassed the effect of different anti microbial agents on dry matter intake and nutrient utilization in goats.

5.1 METABOLITES IN THE FOREGUT OF KID AFTER BIRTH

5.1.1 pH

The stomach of the newborn kids was acidic. Upon perusal of Table 4.1, it was evidenced that initially the pH of stomach is very low (5.04). As a result of milk consumption and by virtue of secretion from the cells of foreguts, the initial pH was acidic. With the reduction of milk consumption and incorporation of solid feeds, the acidity of foregut reduced and gradually moved towards alkaline side. On 2nd week the pH was 6.23 and reached to 6.65 at the age of 4th week. Further with advancement of age, the pH of foregut / rumen increased towards alkaline side and reached up to 6.90 at the age of 9th week. The alkaline saliva copiously secreted into the mouth during feeding continuously poured in to the rumen kept the rumen pH at a levels of 6.0 to 6.7 (Hobson, 1988). The buffering actions of saliva partially neutralized the acid wastes produced from the anaerobic microbial fermentation. The pH of strained rumen liquor of goats fed different forms of complete diets ranged from 6.68 to 7.01 (Samanta et al., 2003). However, feeding of guinea grass or trispecific hybrid to adult local goats represented higher levels (7.30 to 7.40) of rumen pH because of either higher secretion of alkaline saliva or nature of feedstuff itself (Misra et al., 1996). In the present experiment, the rumen pH at the age of 8th to 9th week was comparatively lower than earlier reports because of the diet given to goats.

5.1.2 Ammonia nitrogen

The rumen ammonia nitrogen concentration represents the balance between the degradation of nitrogenous compounds (including protein and non-

100ml. From the age of 7th week onwards, the anaerobic microbes pick up their full function for the degradation of complex polysaccharides for the production of volatile fatty acids. Bomba and Zitnon (1992) observed a similar increase in total VFA during 5th to 11th week of age (except eight week) with a level to that of adult animals reached at 11 week of age. By the presence of vast hoards of microbial population in the rumen, ruminants like goat, sheep, cattle and buffalo ferment the plant structural carbohydrates in to volatile fatty acids for deriving energy. Though the concentration of volatile fatty acids is highly dependent on the nature and type of diet, however, it generally varies from 60 – 120 m mol / liter (Houtert, 1993). Reports suggested that total volatile fatty acids concentration ranged from 9.57 to 11.55 m mol / 100 ml SRL in *Barbari* goats maintained on concentrate, *Leucaena* leaves and oat hay (Senani and Joshi, 1995). In buffaloes, Santra et al., (1996) noticed the total volatile fatty acid concentration 12.65 m mol / 100 ml SRL after 4 hour of post feeding. In the present investigation, the total volatile fatty acid concentration 15.42 m mol / 100 ml SRL at 9th week of age might be due to better quality of diet offered to goats.

5.2 ESTABLISHMENT OF RUMEN MICROBES

The gut ecosystem of ruminants consists of a dense micro-flora. The microbial communities in the gastro-intestinal tract of ruminant have a crucial standing. Bacteria, protozoa and fungi mainly establish this microbial ecosystem. The ruminants achieved the ability to digest the fibrous feed material only with the aid of microbial communities. An adult ruminant can

easily degrade fibrous feed but the young ones are unable to digest fibrous feed only because of the absence of microbes in the foregut. The gastro-intestinal tract of the newly born ruminant is completely sterile (Cunshine et al., 1981) but when it comes in contact with its surroundings, adult animals or contaminated feed, the kid is inoculated with microbes which colonize the gastro intestinal tract. The stomach of a young ruminant is not developed and can not provide conducive environment for growth and multiplication of anaerobic bacteria, protozoa and fungi. With the development of stomach and provision of reducing anaerobic environment, the emergence of microbes starts. The adult rumen remains the chief source of rumen flora for the newborn. The anaerobic conditions of rumen favour the establishment as well as the performance of the rumen microbes.

5.2.1 Bacteria:

Upon perusal of Table 4.2, it was evidenced that bacteria were found just after birth (3.86×10^{10}/ml) which was in agreement with the report of Fonty (1984), Fonty et al., (1984, 1986), who noticed that stomach contained various types of bacteria with the population 10^9 /ml just two days after birth. Anderson et al. (1987) observed the cellulolytic bacteria at an early age of 3 days. Similarly metabolic group of bacteria appeared at the age of 4 days after birth Fonty (1984). In the present investigation, the complexity of rumen micro-flora increased with the advancement of age and therefore confirmed the findings of Muller et al. (1984). Similar to the present findings, most significant changes in bacterial population and metabolic activity were recorded between 4 to 6 week

easily degrade fibrous feed but the young ones are unable to digest fibrous feed only because of the absence of microbes in the foregut. The gastro-intestinal tract of the newly born ruminant is completely sterile (Cunshine et al., 1981) but when it comes in contact with its surroundings, adult animals or contaminated feed, the kid is inoculated with microbes which colonize the gastro intestinal tract. The stomach of a young ruminant is not developed and can not provide conducive environment for growth and multiplication of anaerobic bacteria, protozoa and fungi. With the development of stomach and provision of reducing anaerobic environment, the emergence of microbes starts. The adult rumen remains the chief source of rumen flora for the newborn. The anaerobic conditions of rumen favour the establishment as well as the performance of the rumen microbes.

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of age (Sahoo et al., 2005). At the age of 7th and 8th week, it reached to peak (5.89×10^{10}/ml and 6.00×10^{10}/ml respectively). This was corroborated with the findings of Muller et al., (1984) that bacterial flora become more complex in the age of 8th week.

5.2.2 Protozoa:

In the present study, it was observed that protozoa started to appear at the age of 2nd week, which was in agreement with the findings of Pursor and Moir (1959), Eadie (1962), who noticed that the ciliate established as early as nine days of age in lambs. In the 2nd week of age, the total population was (7.04×10^4/ ml), consisting of only (Table 4.3) *Oligotrichs* with higher population of *Entodinia* (6.67×10^4/ ml) and lesser number of *Polyplastron* (0.37×10^4/ ml). In lamb protozoa appeared in the order that *Entodinia* followed by *Polyplastron* (Fonty et al., 1986). *Polyplastron* (5%) appeared with Entodinia (95%) during second week of age. In the third week of age, the total population was (8.28×10^4/ ml), comprising of *Entodinia* (6.76×10^4/ ml), *Polyplastron* (1.11×10^4/ ml), *Eudiplodinium* (0.25×10^4/ ml) and *Diplodinium* (0.16×10^4/ ml) in the same order as noticed by Fonty et al., (1986) i.e. *Entodinia* followed by *Polyplastron* and then *Eudiplodinium*. But Singh et al (1988) indicated that appearance of *Diplodinium* was followed by *Polyplastron*. In the fourth week of age, the total population was 12.52×10^4/ ml, consisting of one new genus *Ophryoscolex* (0.13×10^4/ ml). During 5 to 7th week of age, the population was 13.67×10^4/ ml, 22.95×10^4/ ml and 28.53×10^4/ ml respectively and having similar genus as up to the fourth week age except a new appeared i.e.

Dasytricha in fifth week. This late appearance of *Dasytricha in* agreement with the investigation of Groliere et al., (1980). An amazing change was noted in the population (41.55× 10^4/ ml) of protozoa in eighth week of age with Oligotrichs as well as Holotrichs; with *Entodinia* (32.07× 10^4/ ml), *Polyplastron* (2.32× 10^4/ ml), *Diplodinium* (1.75× 10^4/ ml), *Eudiplodinium* (2.24× 10^4/ ml), *Ohryoscolex* (1.66× 10^4/ ml), *Isotricha* (0.05× 10^4/ ml) and *Dasytricha* (0.46× 10^4/ ml). Cozzi et al. (2002) found both the *Entodinia* and *Holotrichs* in the rumen sample at the age of 10^{th} week. In ninth week of age, a drop in total protozoa population (37.97× 10^4/ ml) was recorded which were in agreement with the findings of Fonty et al., (1984, 1986c). Natural defaunation occurs in between two to four months after birth (Petkov and Enev, 1979). Santra and Karim (1999) also reported that the *Entodinium* first appeared in the rumen of goat and sheep. *The Entodinia species E. minimum, E. simplex and E. elongatum* appeared first and later on *E. caudatum, E. bursa* (Singh et al., 1988). Thus it was thought that the sequence of protozoa establishment occurred in the order of *Entodinia* > *Polyplastron* > *Eudiplodinium* > *Ophryoscolex* > *Dasytricha* > *Isotricha.*

5.2.3 Fungi:

From Table 4.2, it was clear that fungi appeared during very early age of life. These were present in the foregut fluid of kids even in the first week of age but at very lower concentration (0.21×10^2/ml). Early appearance of fungi was in agreement with the findings of Orpin (1983/84) who suggested that the fungi may appear in the rumen before starting the ingestion of solid feed. Fonty et al.,

(1987) also observed gut anaerobic fungi at 8 -10 days after birth in young lambs. The fungal population increased from third week (8.27×10^3/ml) and reached to peak (14.63×10^3/ml) during 8^{th} week of age. Therefore, the above results confirmed the earlier findings of Orpin (1989) who also noticed the appearance of anaerobic fungi in first 2 week of age and the population reached at peak within 6 to 8 week of age.

5.3 CHARACTERISTICS OF ANAEROBIC FUNGAL ISOLATES

Based on morphology of the anaerobic fungus viz.; rhizomycelial growth, sporangium development, zoospore flagellation, the fungal isolates were categorized into five namely GR_1, GR_2, GR_3, GR_4, and GR_5 (Table 4.4). These isolates were identified according to the classification described by Theodorou, et al. (1996). From the Table 4.4, it can be visualized that mono-centric fungi were predominating in the rumen of local goats. Trinci, et al., (1994) also classified fungi into mono-centric and polycentric on the basis of number of sporangia developed from the thallus. The sporangium of isolate GR_1 was circular, possessed un-segmented rhizomycelia. The released zoospores were mono-flagellated. Thus this isolate was classified as *Piromyces spp.* Earlier workers also classified their anaerobic fungal isolate as *Piromyces spp.* (Orpin 1977, Gold, et al., 1988, Li, et al., 1990, Ho, et al., 1993) based on the above features. Similarly the isolate GR_2, GR_3 and GR_5 were appeared to be *Piromyces* spp. Isolate GR_4 with pear shaped sporangia, unsegmented rhizomycelia and with monoflagellated zoospore seemed to be *Caecomyces* spp.

5.4 METABOLIC PRODUCTS OF FUNGAL ISOLATES

The fermentative end products of anaerobic fungi can be influenced by a variety of cellular and environmental factors including microbes itself. Table 4.5 indicated that all the isolates (GR_1, GR_2, GR_3, GR_4 and GR_5) isolate produced acetate and lactate while metabolizing the cellobiose. Out of these five isolates, GR4 and GR_5 produced butyrate also along with the above metabolites from cellobiose. No propionate and ethanol was detected in the growth media of those five isolates. The anaerobic fungi convert hexose to formate, acetate, ethanol, carbon dioxide and hydrogen (Bauchop and Mountfort, 1981, Lowe et al., 1987b and Bornneman et al., 1989).

5.5 FIBER DEGRADING ENZYMES OF FUNGAL ISOLATES

In the present study, it was observed that all the five isolates secreted endoglucanase (2.852 mIU/ml, 6.229 mIU/ml, 5.400 mIU/ml, 5.204 mIU/ml and 5.825 mIU/ml respectively). FP – ase was found only in GR_2, GR_3 and GR_5 (3.208 mIU/ml, 3.004 mIU/ml and 6.595 mIU/ml). Xylanase was found in GR_1, GR_2 and GR_5 (0.0134 IU/ml, 0.007 IU/ml and 0.024 IU/ml respectively), which was in agreement with the result of Sijtsma and Tan (1993) that *Piromyces* isolate secreted the four enzymes, β-1,4 glucanase, β-xylosidase, β -xylanase and β -endoglucanase. Samanta et al (1998) also detected higher xylanase activity in *Piromyces spp* isolated from the rumen of buffalo.

5.6 FERMENTATIVE ROLE OF PROTOZOA AND FUNGI

Although the protozoa population is comparatively lower than bacteria in the rumen but they constitute about 40-80 % of microbial mass (Harrison and

McAllan 1980). Protozoa plays a significant role on fiber degradation. The role of protozoa and fungi was studied in goat with application of antimicrobial agents.

5.6.1 Rumen metabolites:

There was no significant difference in pH of faunated (6.91), defaunated (6.87) and fungus free (6.64) goats. This was corroborated with the findings of Nangia and Rita (1994). However, some workers noticed in the reduction of rumen pH following defaunation (Pal et al., 1998; Chaudhary, 2002; Sahoo, et al., 2005). The concentration of total VFA reduced (from 14.32 m mol/100 ml to 13.82 m mol /100 ml) in goats on defaunation and remained almost identical (14.53 m mol / 100 ml SRL) during removal of anaerobic fungi. This indicated that probably protozoa might have greater role in rumen than fungus on total volatile fatty acids production. This result was similar to the findings of Kobayashi et al., (1991), Vendrak, et al., (1992) and Santra et al., (1996) who also noticed the reduced concentration of total volatile fatty acids after defaunation. The present investigation contradicted to the results of Sahoo et al., (2005) who recorded higher total volatile fatty acid concentration in the rumen following defaunation.

The concentration of rumen ammonia nitrogen reduced (from 19.71mg/100 ml to 15.15mg/100 ml) significantly upon defaunation. The removal of fungus also reduced the ammonia nitrogen only by 1.2 mg / 100 ml SRL. That clearly defined higher role of protozoa at rumen than the anaerobic fungus. The suppression of bacteria substantially reduced the ammonia nitrogen

concentration. Thus the findings were in line with the results of Eugene et al., (2004). The levels of ammonia nitrogen reduced with lower ciliate population (Pal, et al., 1998, Ivan, et al., 2003) as ciliate have proteolytic activity (Punia et al., 1981, Koenig et al., 2000). On perusal Table 4.7, it was clear that the concentration of total nitrogen reduced (from 64.63 mg/100 ml to 60.3 mg/100 ml) on defaunation. This was in agreement with the report of Nangia and Sharma (1994) that total rumen nitrogen reduced on defaunation. However, Chaudhary et al. (1995) noticed no effect of defaunation on total nitrogen content in strained rumen liquor. The concentration of NPN reduced (from 35.11 mg/100 ml to 31.61 mg/100 ml) on dafaunation (Table 4.7). As non ammonia non protein nitrogen accumulation was greater with protozoa (Hino and Russel, 1987). On perusal Table 4.7, it was clear that there was no effect of defaunation on TCA precipitable nitrogen concentration; however, Chaudhary et al. (1995) noticed increased TCA precipitable nitrogen on defaunation

5.6.2 Microbial population:

On perusal of table 4.8 it was visualized that defaunation significantly affect on fungal and bacterial population. On defaunation the bacteria count become higher (from 21.87×10^{10}/ml to 36.83×10^{10}/ml) in adult goat, which was in agreement with the reports of Nangia and Rita (1994) and Chaudhary (2002). Coleman and Sandfort (1979) suggested that *Entodinia spp.* and large rumen protozoa predate rumen bacteria. The numbers of bacteria in defaunated animals were greater than in normal faunated animal (Bryant and Small, 1960) because

protozoa like *Entodinium caudatum* and *E. simplex* used bacteria as their main source of nitrogen (Bonhomme-Florentin, 1974, Coleman, 1975, 1979, 1980). Defaunation resulted in higher bacterial growth, because of decreased competition for the available feed and decreased predation by ciliates (Eadie and \Mann, 1970, Eadie and Gill, 1971, Demyer and Von Nevel, 1979, Demeyer 1981, Kayouli,ey et al., 1982, Orpin and Letcher, 1984).

Similarly, the fungal population increased (from 24.83×10^2/ml to 36.25×10^2/ml) on defaunation. Defaunation increased fungal zoospores as these were also predated by rumen protozoa (Orpin, 1981). This result was in agreement with the findings of Mathieu et al., (1996) that number of fungi and total bacteria were lower after inoculation with protozoa.

5.6.3 Nutrient utilization

The ciliates are proteolytic and they increase the proteolytic activity of rumen fluid and have active role on rumen protein degradability. On perusal of Table 4.9, it was clear that on defaunation, the dry matter intake in terms of % body weight increased (from 2.24 to 2.85 %) which contradicted to the report of Chaudhary and Srivastava (1996) who found no effect on dry matter intake on defaunation in buffaloes. The removal of fungus also significantly increased the dry matter intake than the control. Defaunation reduced the organic matter digestibility from 70.43 to 65.66 % and in case of removal of fungus the organic matter digestibility was 64 %. But this reduction in the digestibility was found to be non-significant. Rowe et al. (1985) observed a slightly reduced apparent digestibility of OM following defaunation. Defaunation or removal of fungus

has no significant effect on the digestibility of neutral detergent fiber, acid detergent fiber, crude protein, ether extract. The digestibility of crude fiber and nitrogen free extract was reduced by 2 – 4 % following defaunation or removal of fungus. The above findings were identical to the observations of Santra, et al., (1994). The cellulose degradation activities reduced on defaunation. The protozoa perform to one third of fiber breakdown in the rumen (Demeyer, 1981) and carboxymethyl cellulase activity was lower in defaunated animal (Santra, et al., 1996). In the absence of ciliate, the cellulose breakdown decreased as in sheep (Kayouli, et al., 1982) but the present investigation did not record any significant effect of defaunation on the digestibility of neutral detergent fiber.

The rumen microbial population has enormous potential for fiber digestion. Rumen, habouring all microbial population is an excellent fermentative chamber. The exact role of microbe can be identified in their absence. On removing these microbes the concentration of various rumen metabolites changed. On removing protozoa (T_2) various parameters NH_3-N, total VFA reduced with increased in bacterial and fungal population. Protozoa have role in nitrogen utilization (Fujihara et al., 2003) in lambs. Removal of protozoa increased the bacterial number. The numbers of bacteria in defaunated animals are greater than in normal faunated animal (Bryant and Small, 1960). It is due to the predation of bacteria by porotozoa. *Ophryoscolex caudatum* could obtain the amino acids for growth by engulfment of rumen bacteria (Coleman and Reynold, 1982). Thus protozoa help in the production of energy utilization by the host and also involved in orientating the ruminal fermentations. Fungi

are found attached with the fibrous plant particles of digesta as anaerobic fungi penetrate and preferentially colonize tissues traditionally regarded as resistant to degradation, such as recalcitrant sclerenchyma and vascular tissue (Akin, et al., 1989, Bornneman et al., 1990).

Bacteria are the most abundant in rumen. Likewise protozoa and fungi, bacteria are also essential for fiber digestion in the rumen. On comparing the parameters in animals with (T_1) and without (T_4) bacteria (Table 4.7, 4.8 and 4.9), the bacterial significance was reflected in the rumen digestion. Bacteria has an significant role in nutrient utilization, their removal decreased the crude protein intake, digestibility of dry matter, organic matter, DM, OM, NDF, ADF, CP CF, EE, NFE, in compare to bacteria containing animals. As rumen bacteria contain amylase for starch digestion (Cheng, et al., 1990), and these also digest cellulose and hemicellulose (Kamra, 1999). Thus, the present result is agreed that bacteria took major role in rumen fermentation followed by protozoa and fungi.

Chapter 6

Summary and Conclusion

6. SUMMARY AND CONCLUSION

The goat is ideally suited for small and marginal farmers. The local breed of Bundelkhand is black, medium body sized, Roman nose, long ear and thrive well on bushes and shrubs.

A new born kid is just like monogestric animals i.e. with simple undivided sterile stomach completely free from anaerobic microorganisms. But with the growth, all four chambers viz. rumen, reticulum, omasum and abomasums developed and the former two are the growth and multiplication of anaerobic bacteria, protozoa and fungi. The rumen is an unusual environment for microorganisms. Dietary plant complex polymeric nutrients viz. carbohydrate, protein, lipids etc. are generally degraded by rumen microorganisms into characteristics end products which in turn provide nutrients for metabolism by the host. The quantity and quality of rumen fermentation depends upon the types and activities of microorganisms in the rumen.

The present study was divided into two phases. The first phase was the experiment on six new born kids to know the date of establishment of various microbial communities.

The second phase was conducted on twelve adult male local goats which wee divided into four groups viz.T_1 (control), T_2 (defaunated), T_3 (fungus free) and T_4 (bacteria free). The main aim to conduct this part is to know the fermentative role of different groups of microorganism in goat.

The silent achievements of the present investigation are summarized as follows:

6.1 METABOLITES IN THE FOREGUT OF KID

The pH of foregut varied from 5.04 to 6.9 from birth to ninth week which gradually moved towards alkaline side with the advancement of age. The ammonia nitrogen concentration ranged from 10.71 mg/100 ml to 29.55 mg/100 ml SRL. This indicates that activity of proteolytic enzymes increased with the age. The total volatile fatty acid concentration also increased from birth to ninth week of age and varied from 5.38 meq/100ml to 15.42 meq/100ml.

6.2 ESTABLISHMENT OF MICROBES

The bacterial population appeared just after the birth and varied from 3.86×10^{10}/ml to 6.00 $\times10^{10}$/ml till ninth week of age. The protozoa started to appear at the age of second week (7.04×10^{4}/ml) and reached to higher level during 8th week (41.55×10^{4}/ml). Entodinia was the first and most dominant genus. The fungi also appeared in 1st week of age with very low (0.21×10^{3}/ml) and become highest (14.63×10^{4}/ml) in 8th week of age.

Monocentric fungi were predominating in rumen of local goats. Based on the number of flagella in zoospore of selected fungus these were classified as GR_1 GR_2, GR_3, GR_4 and GR_5 out of which GR_4 was *Caecomyces spp.* and rest were *Piromyces spp.*

The GR_1 GR_2, and GR_3, isolates produced only acetate and lactate whereas GR_4 and GR_5 produced acetate, lactate and butyrate. GR_1 secreted endoglucanase

and xylanase, GR_2 and GR_5 secreted endoglucanase, xylanase and FP-ase, GR_3 secreted endoglucanase and FP-ase and GR_4 secreted endoglucanase.

6.3 EFFECT OF ANTIMICROBIAL AGENTS ON RUMEN METABOLITES

There was no effect of antimicrobial agents on pH valves of rumen of different treatment groups as it was similar 6.91 (T_1), 6.87 (T_2), 6.64 (T_3) and 6.95 (T_4). The total volatile fatty acid concentration reduced from 14.32 meq/100ml to 13.82 meq/100 ml on defaunation in (T_2) and on removing bacteria from 14.32 meq/100ml to 7.53 meq/100ml in (T_4) groups but almost similar on removing fungi in (T_3) group. Thus, it is clear that the removal of protozoa and fungi did not affect the total volatile fatty acid concentration as the role was taken by enhanced bacterial population. On defaunation the concentration of ammonia nitrogen decreased from 19.71 mg/100 ml to 15.15 mg/100ml and it reduced by 1.2 mg/100ml on removal of fungi. It clears the higher role of protozoa than anaerobic fungus in rumen. The suppression of bacteria reduced its concentration from 19.71 mg/100ml to 10.53 mg/100ml. The removal of protozoa and fungus slightly reduced the concentration of total nitrogen whereas it greatly reduced on removal of bacteria from 64.63 mg/100ml to 40.57 mg/100ml. The concentration of non protein nitrogen reduced on removal of microbes as from 35.11mg/100ml (T_1) to 31.61 mg/100ml (T_2), 29.84 mg/100ml (T_3) and 13.36 mg/100ml (T_4).It evidences the greater role of microbes in protein degradation. The valve of TCA ppt. nitrogen was not affected by defaunation but increased on removal of fungus from 28.52 mg/100ml to 30.73 mg/100ml and decreased by removing bacteria 28.52 mg/100ml to 27.21 mg/100ml.

6.4 EFFECT OF ANTIMICROBIAL AGENTS ON RUMEN MICROBES

The bacterial population increased on defaunation from 21.87 $\times 10^{10}$/ml to 36.83 $\times 10^{10}$/ml and on removal of fungus from 21.87$\times 10^{10}$/ml to 32.53$\times 10^{10}$/ml. As protozoa predate rumen bacteria. The total protozoa population increased on removing fungus from 4.46 $\times 10^{5}$/ml to 8.2 $\times 10^{5}$/ml whereas a slight increase was found on removing bacteria (5.41$\times 10^{5}$/ml). The fungal population was also increased on defaunation from 24.83 $\times 10^{2}$/ml to 36.25 $\times 10^{2}$/ml and on removing bacteria (34.0 $\times 10^{2}$/ml). As fungal zoospores are predated by protozoa.

6.5 EFFECT OF ANTIMICROBIAL AGENTS ON DMI AND NUTRIENT DIGESTIBILITY

Defaunation or removal of fungus has no significant effect on the digestibility of neutral detergent fibre, acid detergent fibre, crude protein, ether extract. The digestibility of crude fibre and nitrogen free ether extract was reduced by 2.4 % following defaunation or removal of fungus. The removal of bacteria (T_4) decreased crude protein intake, digestibility of dry matter, organic matter, DM. OM, NDF,ADF, CP, CF, EE, NFE in compare to bacteria containing (T_1) animals.

The present investigation depict that, the microbial communities are not present just after birth their number gradually increased with the advancement of age and become higher at age of two months in which bacteria are most abundant members. These microbes attack on fibre particles to cleave complex substances into simple ones for the utilization of their host. Thus the study of establishment and the role of microbes may enhance the utility of local goat.

Chapter 7

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